MALARIA MORTALITY AND MORBIDITY IN THE UNITED STATES FOR THE YEARS 1946, 1947 AND 1948[1]

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In previous annual reports including that for the year 1947 (Faust and Hemphill, 1948) the Committee on Statistics of the National Malaria Society attempted to analyze the malaria mortality and morbidity data compiled by state bureaus of vital statistics and by appropriate Federal Agencies for the previous calendar year. The mortality records served to show fairly satisfactorily the cyclic trends of increase and decrease in malariousness in the Continental United States, particularly in the Southern States, and since about 1936 indicated a steady decline from the mid-depression years (4678 deaths for 1933) to a relatively few deaths for 1947. In contrast to the usefulness of the mortality data, the cases of malaria reported by physicians to state bureaus of vital statistics and compiled by the Division of Public Health Methods, U. S. Public Health Service, have not only been incomplete but in many respects have provided a grossly inaccurate picture, since they have frequently been based on clinical estimates or possibly at times mere guess work rather than on actual records compiled from blood-film diagnosis of the malaria parasite. Thus, a particular state might be reported as having several thousand cases of malaria in a particular year, with infection indicated for every county, whereas blood-films examined by competent diagnosticians in the state laboratories provided evidence of malaria in possibly not more than a fraction of one per cent of the films examined.

Because of the difficulty of evaluating the rapid decline in actual malariousness in the previously highly endemic areas on an annual basis, it seemed appropriate to collect the information annually but to wait for a period of five years before making an analysis. In this way a clearer conception of the problem might be obtained. In the meantime it was believed worthwhile to obtain the records of the state laboratories on blood-film diagnosis for the years 1946, 1947 and 1948, for comparison with the morbidity figures furnished by the state bureaus of vital statistics.

It will be remembered that beginning with 1943 the morbidity data began to be loaded with imported malaria cases, due to exposure of military personnel and, to a minor extent, of civilians in theaters of military operations outside the Continental United States. This loading probably reached its maximum in 1945 and 1946 (Faust, Scott and McDaniel, 1947, Faust and Hemphill, 1948). The request sent by the 1949 Committee to the directors of state laboratories included the desire for laboratory information on the total number of cases confirmed by blood-film diagnosis for each of the three years under consideration, viz., 1946, 1947 and 1948, likewise a breakdown into species diagnosed for each year and data as to whether the infection was

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TABLE 1

Summary by States (or Other Political Subdivisions) of Malaria Morbidity and Mortality in the United States for 1946, 1947 and 1948. Morbidity Data Based on Blood-Film Diagnosis Records in Board of Health Laboratories (BHL) and Reports from Bureaus of Vital Statistics (BVS)*

Legend: V = *Vivax* Malaria, F = *Falciparum* Malaria, M = *Quartan* Malaria, U = Mixed or Undesignated Infections.

POLITICAL SUBDIVISION	MORBIDITY																		MORTALITY		
	1946						1947						1948								
	BHL				Totals		BHL				Totals		BHL				Totals				
	V	F	M	U	BHL	BVS	V	F	M	U	BHL	BVS	V	F	M	U	BHL	BVS	1946	1947	1948
Ala.	343	2			345	1,541	87	2			89	449	23				23	319	31	19	15
Ariz.	20				20	236	18				18	47	5				5	15	2		
Ark.	31	5			36	1,340	17				17	1,323		5			5	636	25	28	22
Cal.	638	16	13	110	677	1,224	111	7	8	12	138	153	34	1	4	1	40	52	5	4	1
Colo.	6	2		15	23	86	2			1	3	9	2				2	8	1		
Conn.	117	2	1		120	456	48	1			49	73	2				2	17		1	
Del.	7				7	12							1				1				
D. C.		2	1		3	27			1		1	3						3			
Fla.	277	1		8	286	493	47	1			48	135	10	3		1	14	123	17	7	12
Ga.	268			2	270	583	66				66	173	13				13	95	12	13	2
Ida.				66	66	66				24	24	16				1	1				
Ill.	21			7	28	565	3	1		5	9	208	1	1		5	7	25	13	6	6
Ind.				88	88	344	31				31	49	8				8	10	4	1	2
Iowa	332				332	280	151				151	24	12				12	19			
Kan.	43			1	44	61	38				38	19	8				8	2	1		
Ky.	83				83	329	64				64	126	5				5	67	6	5	2
La.				227	227	745	45	1			46	213	14				14	50	21	10	16
Me.				86	86	88				111	111	13				44	44	4			
Md.				114	114	73				34	34	10				9	9	6	1		
Mass.	Data Not Available					497				69	69	106				9	9	23	1	4	2
Mich.	1,305				1,305	1,306	148				148	148	26				26	26	3	2	2
Minn.	625	4	3		632	999	188	1			189	373	22				22	80			
Miss.	205	1			206	17,387	49				49	914	49				49	120	29	23	12
Mo.	175			24	199	354	81			8	89	139	5			6	11	24	12	8	5
Mont.	27				27	10	6				6	3	2				2	1	1		
Neb.				152	152	157				18	18	18				3	3	2	2	2	
Nev.	6				6	14	5				5	4	2				2	2	1		
N. H.				17	17	17				2	2	1							2	2	
N. J.	60	1			61	931	42				42	99	10				10	36	2	1	1
N. M.	15				15	84	2				2	21						5	1		
N. Y. State.	443		1	521	965	2,063	94		1	124	219	369	12		1	27	40	77	2	1	
N. Y. City.	151			28	179	1,065	21	2	1	1	25	145	9	1		5	15	40	2	2	3
N. C.	53				53	369	20				20	137	1				1	151	8	2	
N. D.	11				11	5	2				2	1									
Ohio				150	150	418				36	36	16				10	10	7	2	2	2
Okla.	30	4	2	1	37	308	10	2		1	13	536	1		1	1	3	401	11	1	6
Ore.				77	77	78				28	28	28				3	3	3			
Pa.	Data Not Available					1	Data Not Available					9	2		1		3		11	9	2
R. I.	149		2	50	201	203	34			4	38	38	11			1	12	10			
S. C.	70	9	9		88	5,933	21	2	1		24	4,808	3	1			4	3,689	17	13	8
S. D.						18						7									
Tenn.	271	6	6	12	295	388	71		1	6	78	207	14			3	17	80	16	8	7
Tex.	60	1	1		62	6,799	11				11	4,856	5	1			6	3,639	61	30	23
Utah				15	15	93					16	46				2	2	2			
Vt.										1	1										
Va.	13			89	102	486	15			7	22	87	5		1	1	7	39	3		
Wash.				29	29	30				13	13	13				3	3	2		1	1
W. Va.	23				23	88	8				8	7	1				1	1	1		2
Wis.	296				296	81	114				114	70	8				8	10	1	1	1
Wyo.						49						9							1		
Total.	6,174	56	39	1,889	8,058	48,780	1,670	20	13	505	2,224	16,258	326	13	8	135	482	9,921	329	206	155

* These data include information received from the U.S. Public Health Service, and various State Health Departments; thus they do not correspond with the official reported statistics.

acquired within the state, in another part of the Continental United States, or elsewhere.

The information obtained has varied widely in its completeness or inadequacy. After compilation it was observed that the basic laboratory information on morbidity, including species differentiation and total cases (or films) diagnosed as positive for malaria, also the cases reported to the state bureaus of vital statistics, together with the mortality data, could be incorporated in one table, whereas additional information requested was not susceptible of such tabulation.

ACKNOWLEDGMENTS

Sincere appreciation is expressed to the directors of laboratories and epidemiologists of the departments of health of each of the forty-eight states and the District of Columbia, the Director of the Bureau of Preventable Diseases of New York City, the President of the Board of Health of Chicago, and Doctor Justin M. Andrews, Scien ist Director, Communicable Disease Center, Atlanta, for their sympathetic and intelligent cooperation. Without their assistance any attempt to compare laboratory-confirmed infections with reported cases would have resulted in failure.

PRESENTATION OF DATA

A considerable amount of valuable information has been compiled. This is presented in Table 1 and in comments on the replies which could not be included in the table, appended as "Comments" alphabetically arranged by political subdivisions.

It will be noted that in 1946 the total cases (or in some instances films) diagnosed in board of health laboratories (BHL, Table 1), consisted of 6,174 *vivax*, 56 *falciparum*, 39 *quartan* and 1,889 untyped or mixed, totalling 8,058, in contrast to 48,780 "cases" compiled and reported by the bureaus of vital statistics (BVS). For 1947, the corresponding figures were: 1,670 *vivax*, 20 *falciparum*, 13 *quartan*, 505 untyped or mixed and 2,224 total (BHL), and 16,258 total "cases" (BVS), while for 1948 these figures were: 326 *vivax*, 13 *falciparum*, 8 *quartan*, 135 untyped or mixed and 482 total (BHL), and total "cases" 9,921 (BVS). In some of the 50 political subdivisions which reported there were identical figures or relatively close consistency in the totals reported by the laboratories and the bureaus of vital statistics for each year. In other replies there was no relationship. This feature of the present report will be commented on in the "Discussion."

COMMENTS ON DATA NOT SUMMARIZED IN TABLE 1

(by political subdivisions, arranged alphabetically)

Alabama. The figures provided for morbidity by the Director, Bureau of Laboratories, Department of Public Health, are based on positive blood films diagnosed in the combined laboratories, not on the number of confirmed cases. It is probable that there is some duplication in films examined. No information is available as to the localities where the infections were acquired.

Arizona. The number of cases confirmed by blood film diagnosis is a matter of record in the Division of Laboratories, State Department of Health, but the Director of Laboratories has "only the verbal statement of the technicians that they

saw only vivax in smears" they examined during 1946–1948. No information is available of the source of infection.

Arkansas. The data provided by the Director, Hygienic Laboratory, State Board of Health, fail to indicate whether they represent films or diagnosed malaria cases and give no information as to where the infections were acquired.

California. Information furnished by the Chief, Bureau of Disease Control, State Department of Health, provides an excellent breakdown into civilian and military cases and localities where the infections were acquired. The total of *confirmed cases for 1946* was distributed as follows: Civilian, 331 (*vivax* 210, *falciparum* 13, *quartan* 10, mixed one, untyped 97); locally acquired 21 (five through blood transfusions), acquired in other states seven, acquired in Latin America six, acquired in war areas overseas 288, source not designated nine; in addition, 237 cases were reported without laboratory information; Military, 446 (*vivax* 428, *falciparum* three, *quartan* three, untyped 12); locally acquired one, in other states 21, acquired overseas 383, source not designated 41; in addition, 210 cases were reported without laboratory information. *For 1947* the figures were as follows: Civilian, 99 (*vivax* 75, *falciparum* seven, *quartan* eight, untyped nine); locally acquired 18 (three through blood transfusions), in other states two, in Latin America 12, acquired overseas 65, source not designated two; in addition, 11 cases were reported without laboratory information; Military, 39 (vivax 36, untyped three); locally acquired three, in other states 28, in Latin America one, source not designated seven; in addition, four cases were reported without laboratory information. *For 1948* the figures were as follows: Civilian, 35 (*vivax* 30, *falciparum* one, *quartan* three, untyped one); acquired locally 14 (one through blood transfusion), in other states one, in Latin America six, acquired overseas 14; in addition, 11 cases were reported without laboratory information; Military, 5 (*vivax* four, *quartan* one; acquired in other states one, acquired overseas one, source not designated three; in addition, one case was reported without laboratory information. Certified deaths for 1946 consisted of three civilians and two military; for 1947 and 1948 they were all civilian.

Colorado. The information provided by the Director of the Laboratory Section, State Department of Health, is complete except that there is no breakdown into civilian and military. All diagnosed infections in 1946 were acquired overseas, whereas in 1947 one case is assessed as local in origin, eight acquired overseas, and in 1948 one is reported as local and seven from overseas.

Connecticut. The Director, Bureau of Laboratories, State Department of Health, reports that "probably all of the diagnosed cases were acquired overseas."

Delaware. The Director, State Board of Health Laboratory, believes the seven cases for 1946 and one case for 1948 confirmed by laboratory examination acquired malaria overseas.

District of Columbia. The Director, Bureau of Laboratories, Health Department, believes two of the laboratory-diagnosed cases acquired infection in other areas in the U. S., and the other two overseas.

Florida. Of the total reported cases, including those with and those without laboratory confirmation, the sources of infection are broken down as follows by the Director, Bureau of Laboratories, State Board of Health: for 1946, local and in

other states 459, overseas 44; similarly for 1947, 121 and 14; and for 1948, 107 and four.

Georgia. The Director of Laboratories, Department of Public Health, states that for 1946, 4 infections were locally acquired and 217 were from overseas; for 1947 one was local and 65 from overseas, and that for 1948 eight of the diagnosed cases were from overseas. The figures provided refer to positive films and not to cases; for 1946 and 1947 the number of laboratory-confirmed cases is not on record but for 1948 "the thirteen positive blood films were from nine reported cases."

Idaho. No breakdown of figures has been provided by the Director of Laboratories, Department of Public Health.

Illinois. The figures were combined from information furnished by the Director of Public Health and the Chief of the Division of Laboratories for the State and separately from the President, Board of Health for Chicago. Of the total cases diagnosed downstate for 1946 *vivax* amounted to four and seven were untyped; downstate for 1947, one was *vivax* and four were untyped; downstate for 1948, all four cases were untyped. The corresponding figures for Chicago were: for 1946, 17, all *vivax*; for 1948, two *vivax*, one *falciparum*, one untyped, and for 1948, one each *vivax*, *falciparum* and untyped. For the respective three years malaria in military personnel, not included in the State and Chicago statistics, was reported as follows: 533, 197 and 19 cases.

Indiana. According to the Director, Bureau of Laboratories, State Board of Health, species differentiation of malaria parasites was not begun until 1947. The records of the Bureau do not indicate the geographical source of infection.

Iowa. The information provided by the Director, State Hygienic Laboratory, indicates that in 1946, 321 of the 332 diagnosed infections were acquired overseas, the remainder locally. The comparable figures for 1947 were 147 and four, and for 1948 were 12 and none.

Kansas. The Director of Laboratories, State Board of Health, states that he has no information from laboratory records but believes that most of the diagnosed specimens were from ex-service personnel.

Kentucky. From records of his laboratory the Director, Division of Bacteriology, State Board of Health, finds that five of the diagnosed cases for 1946, five for 1947 and two for 1948 acquired infection overseas. However, the observation is made that "as soon as the patient has a chill, most Kentucky doctors give him huge doses of quinine, submitting the blood specimen afterwards. . . . Consequently, my records would not give an accurate picture of the incidence of malaria in Kentucky".

Louisiana. The Director, Division of Laboratoreis, State Department of Health, indicates that species differentiation of malaria parasites was not tabulated for 1946. He could provide no information as to the source of infection.

Maine. The morbidity figures obtained from the Director, Diagnostic Laboratory, Department of Health and Welfare, refer to blood films. The number of positive cases was 75 for a representative group of 100 positive blood films in 1946 and 1947. Additional information secured from Doctor Justin M. Andrews, Communicable Disease Center, Atlanta, indicates that for 1946, 82 of the 88 BVS-

reported cases were contracted outside the State, with six not designated; and that for 1947 and 1948 all of the BVS cases were contracted outside the State.

Maryland. There is no information to add to the meager figures for Maryland.

Massachusetts. The Director, Division of Communicable Diseases, Department of Public Health, states that some of the positive films for 1947 and 1948 represent multiple tests. For 1946, one infection was believed to be locally acquired, one in another State and 507 overseas. For 1947, the cases were all allocated to overseas exposure, and for 1948, one in another State and the remainder overseas.

Michigan. Information obtained from the Director of Disease Control, Records and Statistics of the State, is fully documented as to sources of exposure. For 1946, one infection was acquired locally, two in other States, 1301 overseas and one with source unknown; for 1947, three in other States, two in Mexico and 143 overseas, and for 1948, four in other States, 20 overseas and four with source unknown.

Minnesota. The Assistant Chief, Section of Medical Laboratories, Department of Health, has furnished complete details for the diagnosed cases confirmed by blood-film diagnosis. For 1946, 246 cases, for 1947, 57 cases and for 1948, seven cases were examined in laboratories not under the jurisdiction of his laboratory service. For 1946, one case was locally acquired, two were acquired in other states, 549 overseas and 80 with source unknown; for 1947, one in another State, 163 overseas and 25 with source unknown, and for 1948, 18 overseas and four with source unknown. Two cases counted as quartan infection in 1946 also had vivax malaria and the falciparum case in 1947 also had vivax infection. These were not included in the vivax totals.

Mississippi. The Director of Laboratories, State Board of Health, has no information as to the source of infection of the diagnosed cases.

Missouri. The Principal Biologist, The Division of Health, has furnished data only on diagnoses made in the Central Laboratory. There was no information as to the source of infection.

Montana. The Director, Hygienic Laboratory, State Board of Health, has no record on the sources of infection of the diagnosed cases.

Nebraska. The Director of Laboratories, Department of Health, states that the diagnoses were made in several laboratories, that they were practically all vivax infection, that none were locally acquired, one was acquired in another State in 1946 and all others were overseas malaria.

Nevada. The Director, Division of Laboratories, State Department of Health, indicates that no information is available on the source of infection of the diagnosed cases.

New Hampshire. The Director, Division of Communicable Diseases Control, State Board of Health, believes that most of the 17 cases diagnosed in 1946 were recurrences rather than new infections.

New Jersey. The Chief, Section on Bacteriology, State Department of Health, has no information on the source of infection.

New Mexico. The Director, State Public Health Laboratory, indicates that six of the 1946 cases and one of the 1947 cases acquired infection overseas.

New York State. Information obtained from the Director, Bureau of Epidemiology and Communicable Disease Control, State Department of Health, indicates that for 1946, three cases had locally acquired infection ("one was therapeutic malaria and based on indirect evidence the other two were probably acquired abroad"), four were acquired in other States and 958 overseas; for 1947, two were locally acquired (one through transfusion and one therapeutic malaria), four in other States and 213 overseas, and for 1948, two were locally acquired (one a chronic infection of 25 years duration, one therapeutic malaria), one in another State and 37 overseas.

New York City. The Director, Bureau of Preventable Diseases, indicates that 157 of the cases diagnosed in 1946 acquired malaria overseas; in 1947, one was locally acquired and 19 overseas, and in 1948, 14 were acquired overseas.

North Carolina. The Director, State Laboratory of Hygiene, states that "the number which we gave as having vivax infection in 1946 probably represents the number of patients in whom we found parasites in 1945. The number in 1947 does not include any of the patients in whom we found the parasites in 1946." For 1946, 37 cases were designated as locally acquired and 16 acquired overseas; however, the Director feels "quite confident that in 1946 the number given (16) is considerably less than the actual number." For 1947, 19 were locally acquired and one was acquired overseas. For 1948, the one diagnosed infection was locally acquired.

North Dakota. All cases diagnosed were attributed to overseas exposure.

Ohio. The Chief of Laboratories, Department of Health, indicates that the laboratory diagnoses refer to cases and not to positive blood films. No sources of infection are provided.

Oklahoma. Although the Director of Laboratories, State Department of Health, has no information as to the locality in which malaria was acquired, he is "quite certain that most of those found positive in 1946 were veterans".

Oregon. The Director of Laboratory, State Board of Health, provides a breakdown as to the source of malaria for 1946 (12 in the U. S., 65 outside of the U. S.) and for 1947 (9 in the U. S., 19 outside the U. S.).

Pennsylvania. The Director, Bureau of Laboratories, indicates that the request of information by the Committee on Statistics of the National Malaria Society "identifies one of the defects in Pennsylvania Public Health Statistics".

Rhode Island. Information furnished by the Chief, Division of Communicable Diseases, Department of Health, indicates that all of the diagnosed cases for 1946 and 1947 and six for 1948 acquired their infection overseas.

South Carolina. The Director, Division of Laboratories, State Board of Health, has no information as to source of infection.

South Dakota. The Director, Division of Laboratories, State Board of Health, states that "there has never been reported a case originated within the State".

Tennessee. The Director, Division of Laboratories, Department of Public Health, indicates that the total cases not acquired in Tennessee were 279 in 1946, 91 in 1947 and 13 in 1948.

Texas. The Director of Laboratories, State Department of Health, believes that most of the infections diagnosed in his laboratory were acquired overseas. He

states significantly: "We know of no laboratory-confirmed cases which have terminated fatally recently."

Utah. According to the report of the Director, Division of Laboratories, State Department of Health, all diagnosed cases in 1946 and 1947 acquired infection overseas, while one of the two cases in 1948 was supposedly acquired locally.

Vermont. Out of 23 blood films submitted to the Laboratory of Hygiene, Department of Public Health, in 1946 none was positive for malaria; out of 23 in 1947 one was positive; out of eight in 1948 none was positive.

Virginia. The Director of Laboratories, Department of Health, indicates that of the cases diagnosed by blood films in 1946, 132 were locally acquired, 354 overseas; in 1947, 87 were local and 41 overseas, and in 1948, 21 were local and 18 overseas.

Washington. The Epidemiologist, State Department of Health, states that in 1946, 2 of the diagnosed cases were local, the others acquired infection overseas as did all of those diagnosed in 1947 and 1948. The two local cases were probably exposed to mosquitoes infected from out-of-State human carriers, one most likely a veteran.

West Virginia. The Director, Hygienic Laboratory, Health Department, states that in most instances the diagnosed cases acquired infection overseas.

Wisconsin. According to the Director, Board of Health, all of the diagnosed cases of malaria in 1946, 1947 and 1948 "were veterans and no doubt acquired malaria overseas".

Wyoming. Information provided by the Acting Laboratory Director, State Department of Health, indicates that none of the BVS-reported cases of malaria for 1946, 1947 and 1948 were confirmed by blood-film diagnosis in the State Laboratory.

DISCUSSION

In an analysis of this report the mortality data can appropriately be considered first. For the triennium under appraisal there was an appreciable reduction in the total deaths for 1948 as compared with 1946. In none of the previously highly malarious Southern States was there an exception to this general trend, even though the 1947 figure may have exceeded that of 1946 or the 1948 may have exceeded that of 1947. The total reduction for the period approximated 50 per cent, and for 1948 the rate was approximately one per million for the entire United States. Unquestionably some of the deaths reported for these states, as for those where malaria is no longer endemic, resulted from exposure outside the Continental United States, particularly in veterans. Even with this loading the 1948 mortality figure is a very favorable one. Nevertheless, if any considerable proportion of the reported deaths actually resulted from malaria as the primary cause and the disease was acquired in the political subdivision where death occurred, one must conclude that in 1948 malaria was still a matter of considerable public health concern in Alabama, Arkansas, Florida, Louisiana, Mississippi and Texas, and to a somewhat lesser degree in Illinois, Missouri, Oklahoma and South Carolina.

The morbidity figures are not susceptible to similar analysis as a unit, due to the

great variety of methods employed in different political subdivisions in evaluating "cases". In some replies there was a complete analysis, as in that received from California. This deserves particular comment because it includes a detailed accounting of every reported case. Relatively satisfactory replies were also received from a number of other questionnaires. In many instances, however, the replies were inadequate or unsatisfactory for tabular analysis.

The total cases which were diagnosed in board of health laboratories if compared with those reported by bureaus of vital statistics provide evidence of a several-fold discrepancy. For 1946 this difference was six-fold, for 1947 more than seven-fold and for 1948 about 20-fold. There are probably several reasons to account for these inconsistencies. In the first place, blood films of suspected cases were not all diagnosed in official laboratories and unofficial laboratory diagnoses were not all reported to the directors of these laboratories. Secondly, blood films were not always made during active attacks: in other words, suspected cases did not necessarily have parasitemia at the time the film was prepared. Both of these factors would provide for a considerably smaller number of blood-film diagnoses as recorded by official laboratories than the actual number of cases of malaria in the particular subdivision for a particular year. Probably much more significant in providing this discrepancy is the continued practice of some physicians in previously highly malarious areas to report malaria by estimates rather than by actual evidence based on laboratory confirmation of clinical diagnosis. Thus, it is understandable how a combination of these several factors resulted in a widening rather than a narrowing of the differences between these two types of evidence from 1946 through 1948. Moreover, as malaria continues to decline in the United States, it does not seem likely that any concerted attempt will be made to resolve these difficulties. The only conclusions which can be drawn from weighing the information in hand is that in many instances the laboratory-confirmed cases represent only a portion of the total malaria cases in the areas, whereas the "cases" reported to the bureaus of vital statistics are in many instances overestimated and are consequently unreliable as a gauge of the amount of malaria in the United States. Nevertheless, even if the over-estimates were taken at face value, they would indicate that there were slightly less than ten thousand cases of malaria in the United States in 1948, or one-fifth of the amount reported for 1946.

SUMMARY

This report incorporates information obtained from the board of health laboratories of the several political subdivisions of the United States and other agencies responsible for the diagnosis of malaria in the country for the years 1946, 1947 and 1948. The data are presented in tabular form by political subdivisions, comparing laboratory-diagnosed infections with cases reported to bureaus of vital statistics. Malaria mortality data for the 3-year period are placed in adjacent columns. Additional information not readily tabulated, particularly that regarding the source of infection, is summarized for each of the 50 political subdivisions reporting.

The mortality figures show a 50 per cent decrease for 1948 over 1946, with a substantial reduction in all areas of previously high malariousness. While there is an

even greater decrease in the number of cases reported at the beginning and end of this triennium, there is substantive evidence that the officially-reported laboratory-diagnosed cases represent only a portion of the total amount of malaria, whereas the figures compiled by the bureaus of vital statistics constitute an over-estimate. If one were to employ this latter group of data as a basis for gauging the trend during the triennium, the cases of malaria in the United States in 1948 were slightly less than 10,000 compared with 50,000 in 1946.

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SUMARIO

Este reportaje incluye información obtenida de los laboratorios de los departamentos de salud de las diferentes subdivisiones políticas de los Estados Unidos de Norte América y de otras agencias responsables del diagnóstico de malaria en el país durante los años 1946, 1947 y 1948. Los datos se presentan tabulados por sub-divisiones políticas comparando los casos diagnosticados por laboratorio con los denunciados a los Servicios de estadística vital. Datos de mortalidad por malaria en los 3 años del período considerado se colocan en columnas adyacentes. Información adicional, no tabulable con facilidad, particularmente la referente al origen de las infecciones se resume para cada una de las 50 sub-divisiones políticas que se reportan.

Las cifras de mortalidad muestran una disminución del 50% para 1948 sobre 1946 con una reducción substancial en todas las áreas con historia de alta infección malárica. Aunque existe una reducción aún mayor entre el número de casos reportados al principio y al fín del trienio hay evidencia de que los casos oficialmente reportados con diagnóstico de laboratorio representan solamente una porción de la cantidad total de malaria, mientras que las cifras compiladas por los Servicios de estadística vital constituyen una sobre-estimación. Si se fuera a emplear este último grupo de datos como medida de la tendencia de la infección malárica durante el trienio, los casos de malaria en los Estados Unidos en 1948 fueron ligeramente inferiores a 10000 comparados con 50.000 en 1946.

PHYSIOLOGICAL STUDIES IN THE HUMAN MALARIAL HOST

II. Blood, Plasma, "Extracellular" Fluid Volumes and Ionic Balance During Convalescence from Therapeutic *P. vivax* and *P. falciparum* Infections[1,2]

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In a previous publication (Overman, Hill, and Wong, 1949) results were reported of serial measurement of plasma and "available fluid" volumes; of Na, K and Cl concentrations in whole blood, plasma, erythrocytes and urine; of 24 hour urinary volume, body weight, plasma protein concentration, hematocrit, body temperature and parasitemia in 70 patients during the course of active therapeutic *P. vivax* and *P. falciparum* infections. The blood volume, erythrocyte mass, total circulating plasma protein and 24 hour excretions of Na, K and Cl were calculated from these data.

Serial alterations in these same variables during the convalescent course in 44 patients following either spontaneous remission or chemotherapeutic intervention with atabrine, quinine, plasmochin, paludrine or chloroquine are presented here.

METHODS

As previously reported, control determinations of the variables listed above were made prior to infection with malaria as follows: (1) Plasma volume was measured (in fasting subjects) by the T-1824 dye dilution method (Gibson and Evans, 1937). To circumvent the possibility that early dye disappearance or sequestration might be abnormal in these luetic patients, the multiple (5) sample technique was used rather than the single sample method employed previously by Feldman and Murphy (1945). (2) Hematocrit determinations were made using Wintrobe tubes which were centrifuged for 45 minutes at 2300 G. (3) Total blood volumes and erythrocyte mass were calculated from the measured plasma volume and venous hematocrit. (4) The volume of fluid available for the dilution of thiocyanate (SCN) ("extracellular fluid volume") was determined by a modification of the method of Crandall and Anderson (1934). (5) Plasma and whole blood specific gravities were measured by the copper sulfate method of Phillips, et al. (1943). Plasma protein concentration was estimated from plasma specific gravity and the total circulating protein was calculated from this concentration and the measured plasma volume. (6) Simultaneously with the

[1] The work described in this paper was supported by grants-in-aid from the U. S. Public Health Service, Division of Research Grants and Fellowships.

[2] A portion of the data presented here appears as part of an exhibit at the Fourth International Congresses on Tropical Medicine and Malaria, May 10–18, 1948 at Washington, D. C.

[3] The authors gratefully acknowledge the technical assistance of Mrs. Anne C. Bass and Mr. A. K. Davis.

measurements described above, 10 cc. of heparinized[4] venous blood was obtained for ionic studies. Sodium and potassium concentrations were measured in whole blood, plasma and packed erythrocytes by the flame photometric methods of Overman and Davis (1947). Similarly, an aliquot of the 24-hour pooled urine for the preceding day was analyzed. (7) Chloride determinations on blood, plasma, red cells and urine were made using a modification of the method of Volhard (Peters and Van Slyke, 1932).

Following the establishment of "control" values in the luetic patient, each was infected with malaria and serial determinations were made throughout the active clinical phases of the disease (Overman, et al. 1949) and during convalescence following spontaneous remission or chemotherapeutic intervention. On the day of each determination 24-hour urine volume, body weight, blood pressure, heart rate, body temperature and parasitemia were recorded.

RESULTS

As previously reported, repeated analyses of the data fail to reveal significant differences between *P. vivax* and *P. falciparum* infections when average values for the physiological and biochemical alterations occurring during the active clinical phases are compared. This is true whether such values are plotted against (1) the number of days of positive parasitemia, (2) the accumulate number of paroxysms, or (3) the number of hours of fever above 103° F. Since the number of days of positive parasitemia or the number of days following termination of the disease are the least equivocal of these variables, all other data have been plotted against them.

Although multiple determinations of the previously listed variables were made on each day of positive parasitemia, measurements were made less often following termination of the disease. Consequently we have arbitrarily divided the study following chemotherapeutic intervention into three groups: (1) those made on the first to seventh day following termination, (2) those made on the eighth to twenty-first day, and (3) those made between the sixtieth and three hundred sixty-fifth days. Averate data for these groups is summarized in Table 1.

In order to provide continuity with that which was previously reported (Overman, et al. 1949) we have in each graph prefixed a condensation of the data obtained during active clinical malaria. Each point on each graph represents an average of from 17 to 50 cases.

Figure 1 is presented to illustrate the average clinical severity of the diseases in each of the arbitrary groups. It will be seen that following chemotherapeutic intervention the average parasitemia returned promptly toward zero. While 14 patients in the group studied 8 to 21 days following termination were parasite-free at the time of study, six had counts varying between 500 and 8000 parasites per cubic millimeter of blood. All 17 of the patients in group 3 (60 to 365 days following termination) were free of parasites.

Blood Volume: We have previously reported (Overman and Feldman, 1947) that in simian malaria (*P. knowlesi*) the blood volume alterations in other than terminal or near-terminal cases were equivocal. Similarly, Overman, et al. (1949)

[4] We are indebted to Roche-Organon, Inc. of Nutley, N. J. for generous supplies of Liquaemin (heparin) used in this research.

showed (see Fig. 2) that, while variations in whole blood volume occur during the active clinical course of both *P. vivax* and *P. falciparum* infections in humans, there is no consistent, progressive or significant alteration in this variable. Thus, lack of variation in blood volume during convalescence is an expected phenomenon. The slight reduction noted in group 3 may be the reflection of small seasonal variations in blood volume in humans.

Contrary to certain earlier opinions, the plasma volume of both monkeys and humans with active clinical malaria rises in a manner roughly commensurate with the reduced erythrocyte mass. The dilution of the plasma (see Fig. 7 for plasma

TABLE 1

Comparison of average values in convalescent groups

	GROUP 1	GROUP 2	GROUP 3
Number of cases	46	20	17
Days following termination	1–7	8–21	60–365
Parasites per mm.^{3}	8290	1181	0
Whole blood Na in meq./L	98	99	88
Whole blood K in meq./L	42.9	45.4	52.2
Whole blood Cl in meq./L	84	85	83
Hematocrit in per cent	35.4	37.2	45.6
Plasma Na in meq./L	148	150	146
Plasma K in meq./L	4.7	4.7	4.7
Plasma Cl in meq./L	100	102	103
Erythrocyte Na in meq./L	15.9	15.3	15.3
Erythrocyte K in meq./L	105	108	108
Erythrocyte Cl in meq./L	52.6	54.1	56.3
Urine Na in mM/24 hrs	67	140	205
Urine K in mM/24 hrs	52	68	62
Urine Cl in mM/24 hrs	63	139	194
Plasma volume in cc./Kgm.	60	60	49
Blood volume in cc./Kgm.	94	96	91
"Extracellular" fluid volume in cc./Kgm.	292	245	221
Erythrocyte mass in cc./Kgm.	34	36	42
Plasma protein concentration in gms. per 100 cc.	7.1	7.4	7.4
Total circulating protein in gms.	267	290	257
Circulating protein in gms./Kgm.	4.2	4.5	3.7

protein concentrations) which parallels the red blood cell volume reduction due to hemolytic crises ceases with termination of the disease process, but the return of plasma and erythrocyte volumes to normal is a time consuming process. Normal values are not reached, on the average, until late in the convalescent course.

"Extracellular" Fluid Volume (Thiocyanate space): Figure 3 graphically illustrates that the volume of fluid in the body available for the dilution of SCN increases progressively during active clinical malaria and decreases to normal or below following termination of the disease. From data reported elsewhere (Overman, 1946; Overman, 1947; Overman and Feldman, 1947; Overman, 1948; Overman, Bass, Davis and Golden, 1949; Overman, Hill and Wong, 1949) it would appear that there is serious

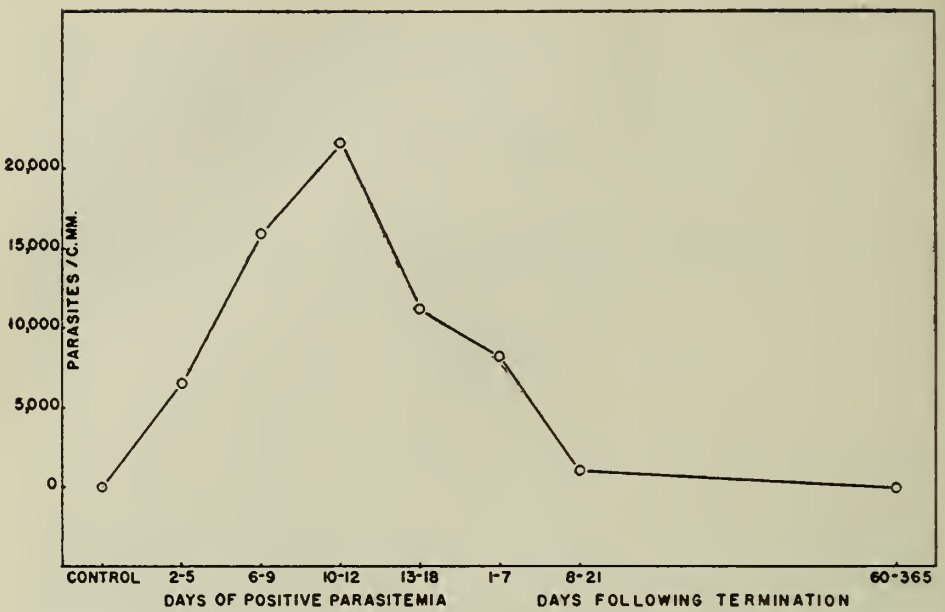

Fig. 1. Average parasitemia of patients measured at intervals during the course of malaria and of convalescence.

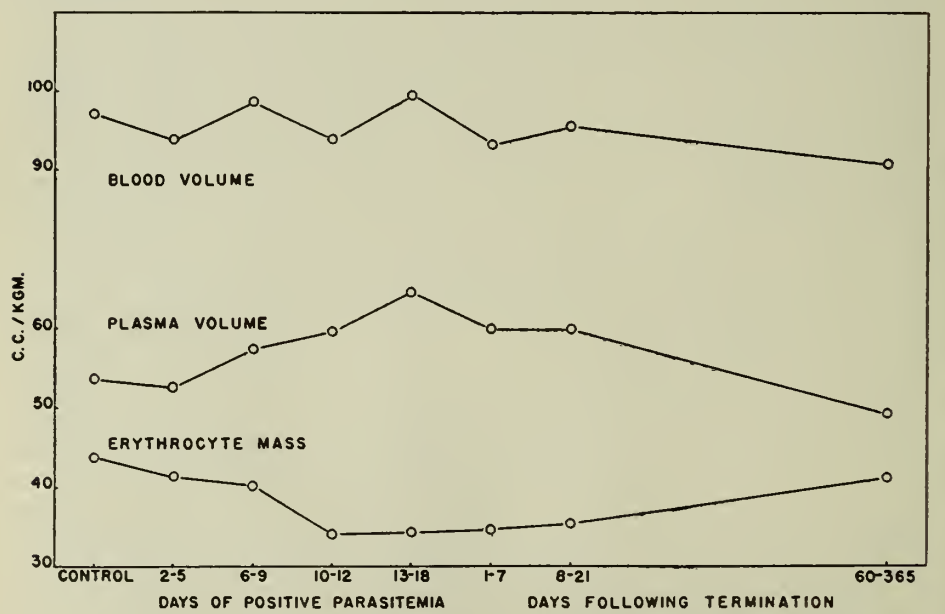

Fig. 2. Variations in blood, plasma, and erythrocyte volumes in human malaria and following its termination (each point represents the average of 17–50 cases).

doubt that true expansion of the extracellular fluid compartment occurs in active malaria. Since monkeys with fulminating *P. knowlesi* infections and human patients

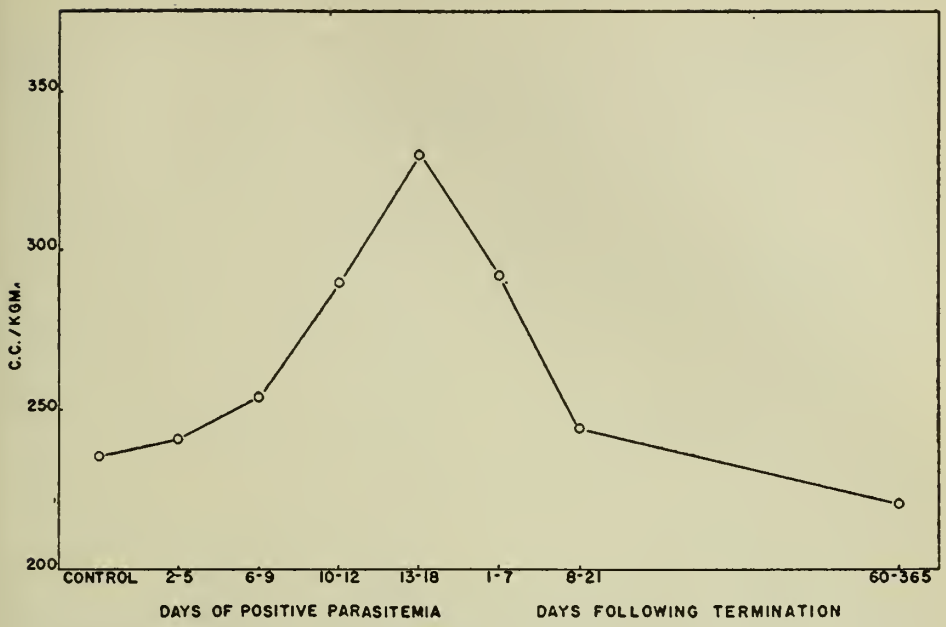

FIG. 3. Showing the increase in the average volume of distribution of NaSCN during malaria and the subsequent decline to normal during recovery.

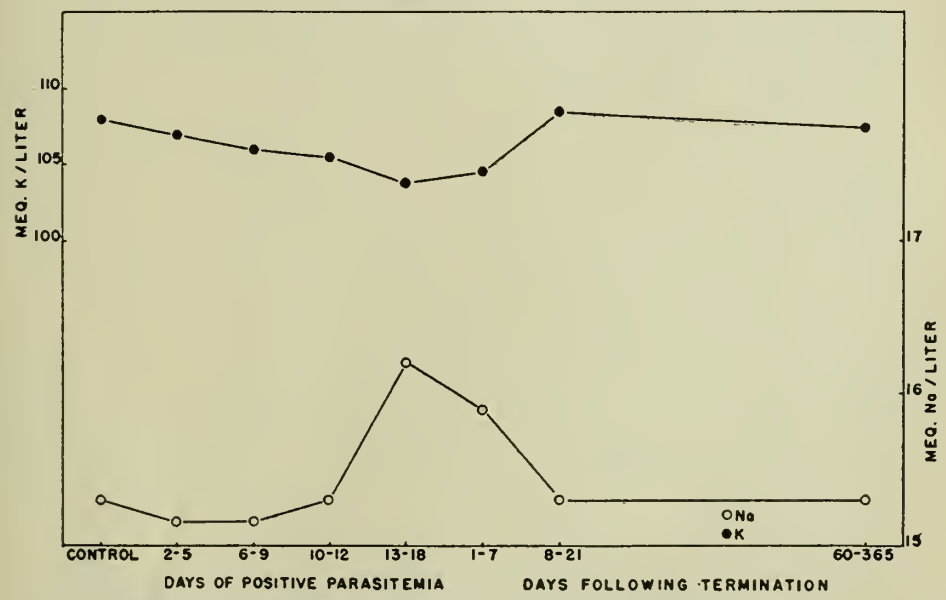

FIG. 4. Changes in the average concentration of Na and K of erythrocytes in malaria and their reversal following termination of the disease.

with severe *P. vivax* or *P. falciparum* show apparent expansions of the extracellular fluid volume to the point of being equal to the calculated total body water, the inter-

pretation of this phenomenon in terms of altered tissue cell permeability to SCN is almost inescapable.

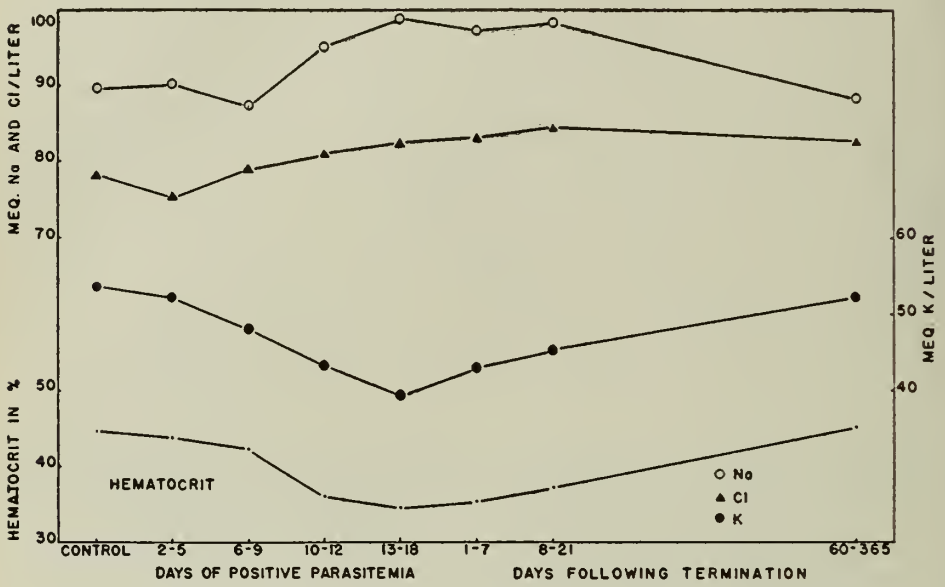

FIG. 5. Alterations in whole blood ionic concentrations with changes in the hematocrit.

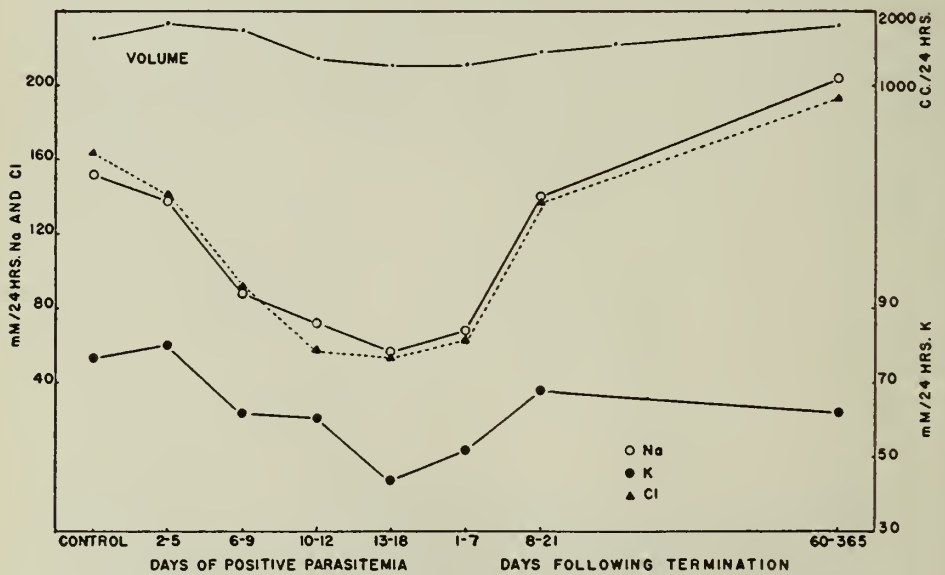

FIG. 6. Urinary excretion pattern of Na, Cl and K in human malaria and during recovery.

Erythrocytic Ionic Levels: This hypothesis of increased permeability of cells is confirmed by measurement of erythrocytic Na and K levels. The increase in Na and

decrease in K seen during the course of the disease are reversible and return to normal levels during the second and third weeks following termination (Fig. 4). Although these average changes fail to be statistically significant, individuals who were more severely ill showed an increase of as much as 100 per cent in erythrocyte Na and a subsequent gradual return to normal levels (Overman et al. 1949, Fig. 10).

Whole Blood Ionic Levels and Hematocrit: Whole blood ionic levels fail to reveal cellular shifts since they merely reflect changes in the hematocrit (Fig. 5). As the percentage of erythrocytes falls, the blood K level falls also. The increase in blood Na and Cl levels reflects the increased percentage of the blood that is plasma. As the hematocrit slowly returns to normal during convalescence, the blood K also rises and the Na falls to normal concentrations.

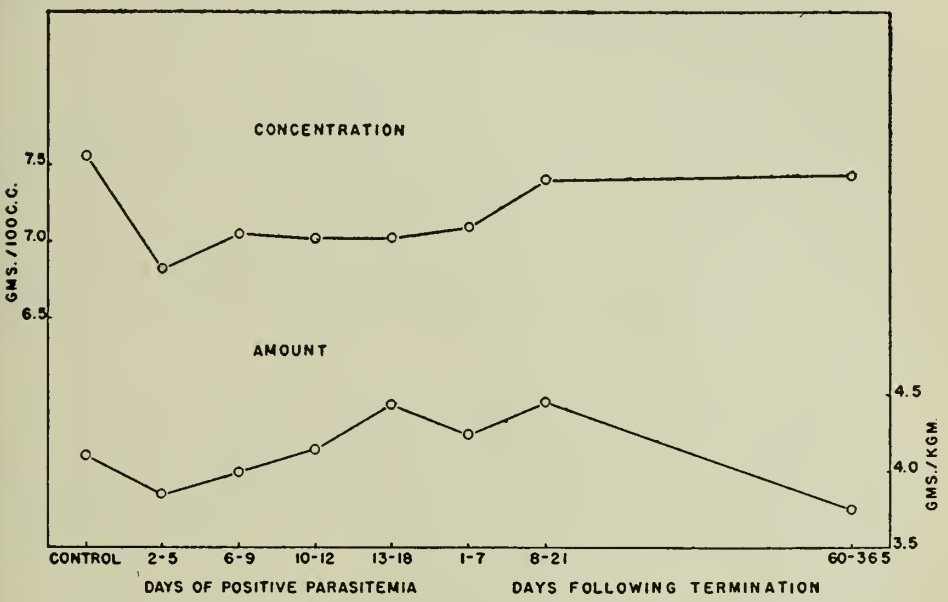

FIG. 7. Average changes in the concentration and total amount of plasma protein.

Urinary Excretion of Ions: Since the plasma levels of Na and Cl remain constant during the course of the disease (Table 1), an entrance of Na and Cl into cells and exit of K from them should cause a decrease in the amounts of Na and Cl excreted by the kidney. Figure 6 shows such a decrease and a subsequent return to the normal pattern by the second period of convalescence. Urinary K excretion also falls during malaria, but to a lesser degree, and rapidly returns to normal levels following termination.

Plasma Proteins: Although the concentration of plasma proteins falls during the course of human malaria, there is actually an increase in the total amount of circulating protein per kg. of body weight. This increase reflects the expanded plasma volume, whereas the fall in concentration indicates that more water than protein has been added during the dilution process. Figure 7 shows that the amount of protein per kg.

falls gradually to normal with the reduction of the plasma volume during recovery and that the concentration rises concommittantly.

DISCUSSION

Many of the problems arising in the interpretation of these data, particularly those changes occurring during active clinical malaria, have been discussed previously (Overman, Hill and Wong, 1949). It has been pointed out that parasitic destruction of erythrocytes, which if uncompensated would rapidly lead to oligemia and circulatory failure, evokes dilution of the blood with tissue fluid. Toxic products of parasite metabolism or, more probably, the effects of parasite metabolism in depressing host adrenal cortical activity bring about changes in cell membrane permeability, such that the cells which were previously impervious to the accumulation of Na or the loss of K are now confronted with these alterations in chemical anatomy. The resulting tendency for reduction in the extracellular concentration of Na and Cl and rise in K concentration is largely offset by renal action.

Following spontaneous remission or chemotherapeutic intervention, each of the observed biochemical or physiological alterations returned to normal. However, it must be pointed out that such biochemical reconstitutions did not all occur at once and in all instances outlasted "clinical convalescence".

Certain minor differences in recovery time were noted between *P. vivax* and *P. falciparum* during the convalescent period. In general, however, no significant qualitative and but few quantitative physiological or biochemical differences in host reactivity to the two diseases were found.

SUMMARY

1. Serial measurements of blood, plasma, "available fluid" and erythrocyte volumes; of Na, K and Cl concentrations in whole blood, plasma, erythrocytes, and urine; of 24 hour urinary volume, body weight, plasma protein concentration, hematocrit, and parasitemia in 44 patients following spontaneous remission or chemotherapeutic intervention in the course of *P. vivax* and *P. falciparum* infections are reported.

2. Data are presented to show that the increased "available fluid" volume, the increased erythrocyte Na, and the decreased erythrocyte K return to normal during the second and third weeks of convalescence, indicates reversibility of altered cell permeability.

3. Whole blood ionic concentrations, plasma protein concentration, total amount plasma protein and plasma volume data reveal a slower return to normal with the rebuilding of the red cell mass.

4. The urinary excretion pattern was also shown to return to normal during the second period of recovery.

ACKNOWLEDGEMENTS

The authors are indebted to Mrs. Virginia Fogg and Mrs. Virginia Drinnon and to the facilities of the Malaria Investigations Laboratory of the Department of Health and Safety of the Tennessee Valley Authority in making daily parasite counts on these patients.

The facilities of the Gailor Psychiatric Hospital, Memphis, were used in housing and caring for the patients studied.

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SUMARIO

1. Se presentan una serie de mediciones de sangre, plasma, fluido disponible (available fluid) y volúmenes de eritrocitos; de concentraciones de Na, K y Cl en sangre, plas ma, eritrocitos y orina; de volúmen de orina en 24 horas, peso del cuerpo, concentración de proteinas en el plasma, hematocrito y parasitemia en 44 pacientes en seguida de remisión espontánea o de intervención quimioterápica en el curso de infecciones de *P. vivax* y de *P. falciparum*.

2. Se presentan datos que muestran que el incremento en el volúmen de fluido disponible, el incremento de Na en los eritrocitos y la disminución de K en los mismos vuelven a la normalidad durante la segunda y tercera semana de la convalecencia indicando reversibilidad en la permeabilidad de las células alteradas.

3. La concentración ionica en la sangre total, concentración de proteinas en el plasma, cantidad total de proteinas en el plasma ye volumen del plasma revelan un retorno más lento a la normalidad con la rehabilitación de la masa de glóbulos rojos.

4. El cuadro de excreción urinaria retornó también a la normalidad durante el segundo período del recobro.

THE RESPONSE OF WHITE PEKIN DUCKLINGS INFECTED WITH *PLASMODIUM LOPHURAE* TO INJECTIONS OF PLASMA FROM RECOVERED DUCKS[1]

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Taliaferro and Taliaferro (1940) have demonstrated protective properties against *Plasmodium lophurae* in the serum of recovered and superinfected chickens. Their procedure was first to inject intravenously a number of test chicks with graded amounts of the serum and later inoculate test chicks and controls intravenously with comparable numbers of parasitized chick corpuscles, and inject the serum thereafter at daily intervals. It was mentioned in a previous paper that an attempt had been made to repeat the Taliaferros' work using ducklings throughout instead of chicks, and that failure to alter significantly the course of the infection resulted from the injection of immune duck plasma into the infected ducklings. Trager and McGhee (1949), however, have recently reported that the plasmas of about one-fourth of the adult ducks tested by them showed antimalarial activity when injected into one-week-old ducklings.

MATERIALS AND METHODS

The parasite had been maintained by blood-passaging through ducks at intervals of four to six days. The ducklings were White Pekins purchased from a commercial hatchery, fed a commercial duck feed, and started on the tests when 11- to 14-days-old. The immune duck plasma was prepared by the procedure previously described (Becker, Brodine, Marousek and Byrd, 1949), and in each of these experiments was pooled from two or more ducks which had recovered from *P. lophurae* infection two or three weeks before the plasma was drawn. Since it has been claimed previously (Becker, Brodine, and Clappison, 1949) that demonstrable parasites reappear sooner or later more often than not in the blood of recovered ducks, it should be explained that in no case was the test blood drawn from recovered ducks which at the time showed parasites in the blood smears. All injections were into the leg vein. Plasma was first injected one and one-half hours before the infected duck erythrocytes, and on following days as noted in Table 1. The percents of parasitized cells found on selected days in test and control birds also appear in the table. Age of the ducks when inoculated appears under group designation. The notations concerning amount of plasma and numbers of parasitized cells injected are for each 100 g. of bird weight. The controls received the same volume of physiological salt solution as the tests received of plasma. Blood smears were stained in Giemsa. The method of reading the smears was the same as described in our papers to which reference has already been made.

[1] This investigation was supported in part by Research Grant 605 (c) from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service.

EXPERIMENTAL RESULTS

Series 1. In this series there were four groups: (1) the controls, saline-recipients; (2) receptors of plasma pooled from six half-grown uninfected male ducks; (3) receptors of plasma pooled from recovered halfgrown ducks, five males and four females;

TABLE 1

Mean and standard deviations of percent parasitized cells on selected days in blood of ducks infected with P. lophurae *arranged by groups according to treatment (C = controls injected with physiological salt solution; N = normal-plasma-recipients; I = immune-plasma-recipients; R = rat-plasma-recipients. No. of survivors in parentheses)*

SERIES	GROUP	NO. DUCKS	DATA	DAYS AFTER INOCULATION							
				2	3	4	5	6	7	8	9
	1	8	M		6.9	32	53	73	61	48(5)	42(1)
	(C)		S.D. (±)		1.8	6	6	5	12	5	—
1	2	8	M		6.2	29	42	49	22	4(5)	1(1)
Age, 11 da.	(N)		S.D. (±)		1.1	4	9	21	21	6	—
1.3×10^8 P.C.											
1 cc. plasma on	3	8	M		5.2	31	45	72	66	46(4)	23(2)
days 0–6	(I)		S.D. (±)		0.7	7	7	6	10	21	—
	4	7	M		6.5	31	50	71	47	33	33
	(R)		S.D. (±)		2.0	10	11	14	15	19	29
2	1	6	M	1.1	6.1	19	42	58	67(4)	—	(0)
Age, 13 da.	(C)		S.D. (±)	0.6	1.5	3	13	6	14	—	
1.3×10^8 P.C.											
2 cc. plasma on	2	6	M	1.3	8.6	22	47	66	63	—	49(3)
days 0–6	(N)		S.D. (±)	0.3	1.6	7	12	5	2	—	9
	3	6	M	1.0	6.0	21	43	59	41	—	27
	(I)		S.D. (±)	0.4	2.6	9	19	8	18	—	22
3	1	5	M		7.6	36	73	69	58(3)	47(2)	24(2)
Age, 12 da.	(C)		S.D. (±)		1.9	5	4	7	10	—	—
1.3×10^8 P.C.											
2 cc. plasma on	2	5	M		8.1	39	69	68	61(3)	52(1)	33(1)
days 0–4	(I)		S.D. (±)		2.1	7	5	7	12	—	—
4	1	4	M		0.8	—	12	47	73	86	(0)
Age, 14 da.	(C)		S.D. (±)		0.2	—	3	4	3	4	—
1.0×10^8 P.C.											
2 cc. plasma on	2	4	M		0.5	—	10	31	58	74	42(2)
days 0–5	(I)		S.D. (±)		0.2	—	2	6	5	7	—

and (4) receptors of plasma from grown white rats of both sexes. The rats had recovered from *Trypanosoma lewisi* infection several weeks before they were bled, a fact that is probably immaterial in the present instance.

The mean parasitemias as recorded in Table 1 were fairly comparable in the four

groups on the third, fourth, and fifth days. The same is true for groups 1, 3, and 4 on the sixth and seventh days, but the means of group 2 are lower than the others, that of group 2 being significantly lower than that of the control on the seventh day. The more susceptible ducks in each group died on the eighth and ninth days, leaving only four survivors in the first three groups. Why none of the seven ducks in group 4 succumbed despite heavy parasitemias on the sixth day is an open question.

Series 2. The three groups received (1) saline solution, (2) normal duck plasma from three half-grown normal male ducks, and (3) immune duck plasma from four recovered ducks on both sexes, respectively. Groups 1, 2 and 3 had comparable records on days, 2, 3, 4 and 5, but on day 7 the mean of the receptors of immune plasma was distinctly lower than the others, though not significantly. Strangely enough, none of group 3 succumbed, though all of group 1 and half of group 2 died by the ninth day.

Series 3. Group 2 of this series received the pooled plasma from three recovered ducks. Counts and casualties in the two groups were fairly comparable throughout.

Series 4. Group 2 received plasma pooled from four recovered ducks. The mean counts for group 2 were lower than those of group 1 throughout, and significantly so on the sixth and seventh days.

DISCUSSION

There were in the four series 23 control ducklings injected with physiological salt solution, 23 receptors of "immune" duck plasma, 14 receptors of normal duck plasma, and 7 receptors of rat plasma. Readings of the stained blood smears showed that the course of the control infections proceeded normally, with peaks on days 5–7. Mortality was high in the control birds with only three of 23 surviving after nine days. The course of the infection and the mortality in the 8 receptors of immune duck plasma belonging to group 3 of series 1 were about as close to the same phenomena in the controls (group 1, series 1) as might have been anticipated had both groups been controls. The same statement could apply to the receptors of immune plasma in series 2 (group 3) and series 3 (group 2). In series 4 (group 2), however, the counts for the receptors of immune plasma were significantly lower on days 6 and 7.

A generalization on the effect of normal plasma is difficult. In series 1, the parasitemia was definitely and significantly lower in the receptors (group 2) than in the controls (group 1) on days 6, 7 and 8. Mortality was the same in the two groups. In series 2 the course of the infection was as similar in the receptors of normal plasma (group 2) and controls (group 1) as could have been expected had both groups been untreated. The difference in mortality, which was 100 per cent in the controls and 50 per cent in the receptors of normal plasma, was probably not significant in groups of six each.

It is apparent that differential antimalarial effects of immune duck plasma as compared with physiological salt solution were demonstrated in two out of four experiments, but not in two others. Immune duck plasma injected into chicks uniformly depressed the parasitemia (Becker *et al.*, 1949) and in a much more striking manner. This comparison poses the important problem of the nature of the different types of

response in the two specific bird hosts. The reaction of the infected chicks to even smaller injections of immune plasma than were administered to the infected ducklings proves the presence of antibody in the duck plasma, but the threshold of response is higher in the duck. It is to be kept in mind also that *P. lophurae* infections in chicks ordinarily do not reach the peaks attained in ducks, nor do they relapse with the frequency observed in ducks (Becker, Brodine, and Clappison, 1949). In other words, innate resistance, actively acquired immunity, and response to passively conferred antibody are all of a lower order in the duck than in the chicken.

Trager and McGhee's finding that the plasmas of one adult duck out of four showed antimalarial activity when injected into ducklings means, when stated conversely, that three adult ducks out of four did not show antimalarial activity. When in addition it is considered that in our experiments none of the plasma donors was adult and that the plasma from a number of ducks was pooled, the harmony between our results and theirs becomes more apparent.

SUMMARY

Injections of pooled immune duck plasma into ducklings infected with *Plasmodium lophurae* significantly depressed the parasitemia in two of four tests, but no more so than did injections of normal duck plasma in one of two tests. Since the presence of immune bodies in the plasma of recovered ducks can be much more consistently demonstrated in chick infections, the explanation for the comparative ineffectiveness of such plasma in ducks lies entirely in the nature of the host response.

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SUMARIO

Inyecciones de una mezcla de plasma inmume de un grupo de patos fueron aplicadas a patitos infectados con *Plasmodium lophurae* y bajaron la parasitemia en forma significante en dos de cuatro pruebas, pero no en mayor proporción que lo produjeron inyecciones de plasma de pato normal en una de dos pruebas efectuadas. Puesto que la presencia de anticuerpos en el plasma de patos curados espontáneamente puede ser demostrada mucho más fácilmente en infecciones de pollos, la explicación acerca de la ineficacia de tal plasma en patos, descansa enteramente en la naturaleza del huésped.

THE COMPARATIVE SUSCEPTIBILITY OF *ANOPHELES QUADRIMACULATUS* AND *ANOPHELES FREEBORNI* TO INFECTION BY *PLASMODIUM VIVAX* (ST. ELIZABETH STRAIN)

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Anopheles quadrimaculatus, in the Southeast, and *Anopheles freeborni*, in the far West, have long been accepted as the principal vectors of malaria in the U. S. A. It has been shown that both these species are readily susceptible to infection by *Plasmodium vivax* of foreign origin (Young, *et al.*, 1945, and Young and Burgess, 1948). This report presents comparative data on the susceptibility of laboratory colonized strains of these two anophelines to infection by a domestic strain, St. Elizabeth, of *P. vivax*.

METHODS AND PROCEDURES

A total of 40 comparative feedings were made. During each of these feedings, approximately equal numbers of laboratory-bred *A. quadrimaculatus* (Q-1 strain) and *A. freeborni* (F-1 strain) were fed simultaneously upon a patient infected with St. Elizabeth strain of *P. vivax*.

Prior to the infective blood meal all mosquitoes were given standard pre-feeding insectary treatment (Burgess and Young, 1944). At the time of biting, the *A. quadrimaculatus* were four days old and the *A. freeborni* seven days old, which respective ages seem to be optimum to secure the best feeding.

After the blood meal the mosquitoes were kept under standard conditions of this insectary, 74°F. ± 2° temperature and 60–80 per cent relative humidity.

Water and food were supplied by placing pads wet with two per cent syrup (dark Karo) solution over the bobbinet tops of the cages each night. These pads were removed each morning to prevent fermentation and fungus growth.

Dissections, usually daily, were begun on the sixth day after the infective feeding and were continued until after sporozoites appeared in the glands of the infected mosquitoes.

Suseceptibility to infection was measured by (1) the percentage of mosquitoes that became infected; (2) the actual number of oöcysts on each infected gut dissected; and (3) the numbers of sporozoites in the salivary glands.

RESULTS

Table 1 is a summary of the data gathered from the dissection of 1,770 *A. quadrimaculatus* and 1,468 *A. freeborni* which had fed upon malarious patients.

The percentage of *A. freeborni* infected was greater than that for *A. quadrimacu-*

[1] Acknowledgment is gratefully given to F. N. Hemphill for help with statistical analysis.

TABLE 1

Comparative data on susceptibility of A. freeborni *and* A. quadrimaculatus *to* P. vivax *(St. Elizabeth strain)*

	A. QUADRIMACULATUS	A. FREEBORNI	CRITICAL RATIO*	INDEX FREEBORNI ÷ QUADRIMACULUS
Number mosquitoes dissected	1,770	1,468		
Number mosquitoes infected	1,042	962		
Percent infected and standard deviation	58.87 ± 1.15	65.53 ± 1.26	3.9	1.1
Number mosquitoes examined for oöcyst densities	553	527		
Mean oöcysts per infected gut and standard deviation of mean	17.93 ± 1.28	28.36 ± 1.81	4.6	1.6
Mean oöcysts per dissected gut	10.56	18.58		1.8
Average sporozoite intensity in infected mosquitoes§	2.5	3.0		1.2

* Critical ratio is the observed difference between the means divided by its standard error. Values greater than 2.0 are considered significant.

§ Sporozoites were grouped as follows: 1–9, 1+; 10–99, 2+; 100–999, 3+; and 1000 and over 4+. The sum of these plus signs divided by the total number gives the average, e.g., one 2+ and one 3+ gives an average of 2.5+.

TABLE 2

Oöcyst densities per infected gut in A. quadrimaculatus *and* A. freeborni *when arranged according to gametocytes per cubic millimeter*

GAMETOCYTES PER CMM.	INFECTED MOSQUITOES				OÖCYST INDEX
	Number of guts		Average oöcysts per gut		
	quadrimaculatus	*freeborni*	*quadrimaculatus*	*freeborni*	*freeborni/ quadrimaculatus*
20–29	43	41	4.1	3.7	0.9
30–39	22	18	29.7	57.8	2.0
40–49	18	21	2.2	3.9	1.8
50–59	13	18	3.1	3.8	1.2
60–69	48	25	3.5	5.2	1.5
70–79	89	91	11.0	15.2	1.4
80–89	51	39	5.9	10.0	1.7
110–119	12	19	2.8	5.9	2.2
120–129	21	25	3.3	10.0	3.0
130–139	21	22	7.1	24.7	3.5
160–169	28	30	5.8	15.9	2.7
180–189	46	39	4.2	19.5	4.6
190–199	19	14	24.5	74.3	3.0
200–209	20	20	21.7	79.9	3.7
310–319	13	12	7.1	12.7	1.8
340–349	10	8	10.6	16.9	1.6
400–409	21	18	31.4	40.8	1.3
680–689	21	22	122.1	166.1	1.4

latus. Furthermore, the intensity of sporozoites in the glands was greater in *A. freeborni*.

Not only were there more individuals of *A. freeborni* with oöcysts on the gut but there were more oöcysts per gut than in *A. quadrimaculatus* in the ratio of 1.6 to 1; the difference is significant, having a critical ratio of 4.6.

To determine the relationship of gametocyte density at time of feeding to the resultant infections, the gametocytes per cubic millimeter were divided into intervals of 20 and the intensity of the infections as expressed by oöcyst intensity in each of these groupings was measured. These data are shown in table 2. Since the gametocyte densities could not be controlled, there are some densities for which no figures are available.

By dividing the average number of oöcysts per infected gut of *A. quadrimaculatus* into the average number of oöcysts in *A. freeborni*, an index was obtained (table 2). When this index figure is 1.0, it indicates that there was the same number of oöcysts, when less than 1.0, *A. quadrimaculatus* had the greater number, and when greater than 1.0, the converse was true. Only at the point of lowest gametocyte density, between 20 and 29 per cmm., was the infection greater in *A. quadrimaculatus* than in *A. freeborni* and the difference there was very slight.

In all other groups, *A. freeborni* had more oöcysts per gut. The greatest difference occurred in the groups where the gametocytes ranged between 110 and 209 per cmm.; in this range, the infection in *A. freeborni* was more than twice that of *A. quadrimaculatus*. The highest index was at 180–189 gametocytes per cmm. when *A. freeborni* had 4.6 times as many oöcysts as the other species. When the gametocytes were below or above this range of 110–209 per cmm., the difference in susceptibility was less in evidence.

DISCUSSION

By each method of appraisal, *A. freeborni* was more susceptible than *A. quadrimaculatus* to a domestic strain of *P. vivax*. A similar difference in susceptibility to foreign strains of *P. vivax* malaria has been shown previously (Young and Burgess, 1948). The combined evidence indicates that *A. freeborni* has a greater innate susceptibility to *P. vivax* in general than does *A. quadrimaculatus*.

SUMMARY AND CONCLUSIONS

In a series of 40 comparative feedings involving 3,238 mosquitoes, *Anopheles freeborni* showed a greater susceptibility than *A. quadrimaculatus* to infection by a domestic strain, St. Elizabeth, of *Plasmodium vivax*. Not only was there a higher percentage of mosquitoes infected but the intensity of the infections was greater in the former species.

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SUMARIO

En una serie de 40 comidas infectantes comparativas de *Plasmodium vivax* (cepa St. Elizabeth) efectuadas a un total de 3.238 mosquitos, *Anopheles freeborni* mostró una suceptibilidad mayor que *A. quadrimaculatus* al mentado parásito. No solamente hubo un porcentaje mayor de mosquitos infectados sino que la intensidad de la infección fué mayor en la primera especie.

STUDIES IN HUMAN MALARIA

XXVII. Observations on the use of Pentaquine in the Prevention and Treatment of Chesson Strain *vivax* Malaria

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Pentaquine [SN 13,276 or 6-methoxy-8-(5-isopropylaminoamylamino) quinoline] alone and in various combinations, has been used in a large number of volunteers infected with *vivax* malaria at the Federal Correctional Institution, Seagoville, Texas. Observations in these cases, which supplement studies on the 8-aminoquinoline drugs done during the World War II drug testing program, will be described under the following general headings:

(1) Attempted prophylaxis of Chesson strain *vivax* malaria with pentaquine and quinine, given concurrently for six days after exposure to infected mosquitoes.
(2) Attempted radical cure of Chesson strain *vivax* malaria with pentaquine plus cinchonidine or pentaquine plus cinchonine.
(3) Results with various dosage combinations of pentaquine and quinine in curative therapy.
(4) Toxic reactions, including one instance of severe acute intravascular hemolysis in a white subject.

MATERIAL AND METHODS

All of the studies were carried out in the Federal Correctional Institution, Seagoville, Texas, using methods previously described (Coatney *et al.*, 1948). Inmate volunteers were infected with the Chesson strain of *Plasmodium vivax* by the bites of *Anopheles quadrimaculatus* mosquitoes which had fed upon suitable gametocyte carriers approximately two weeks earlier. After feeding, each mosquito was dissected and the number of sporozoites in its salivary glands observed and graded on a scale of 0 to 4+.

Volunteers remained under observation for at least 18 months, with blood smears being made at least twice weekly except during the last few months of observation when they were made once weekly. During acute attacks of malaria the men were hospitalized, blood smears were made daily, and temperature readings were taken and recorded at least every four hours. Temperatures were recorded at other times if parasitemia or acute symptoms appeared.

The following antimalarial drugs were used: pentaquine phosphate (75 per cent base), quinine sulfate (83 per cent base), cinchonidine sulfate (79 per cent base), and cinchonine sulfate (81 per cent base). All dosages are expressed in terms of base, unless otherwise indicated.

Concentrations of pentaquine in blood plasma were determined by the method of Brodie *et al.* (1947), those of quinine by the method of Brodie and Udenfriend (1943) and those of cinchonine and cinchonidine by the methyl orange procedure of Brodie and Udenfriend (1945).

In the majority of the subjects given pentaquine, hemoglobin determinations and urinalyses were made daily. Complete blood counts and estimations of methemoglobin in whole blood, by an adaptation of the procedure of Evelyn and Malloy (Wendel, 1944), were made every other day.

TABLE 1

Trial of pentaquine and quinine as a prophylactic combination against sporozoite-induced Chesson strain vivax *malaria*

DRUG REGIMEN	SET NO.*	VOLUNTEER NO.	INFECTED MOSQUITOES PER SUBJECT ON DAY 0		PREPATENT PERIOD	INCUBATION PERIOD 101° F
			No.	Sum of pluses†		
					days	*days*
Pentaquine, 60 mgm. per day + quinine,	1	S-1	10	36	21	23
2.0 grams per day, on the day before,	2	S-2	10	38	15	13
on the day of, and for 6 days after the	3	S-3‡	10	39	12‡	13
day of bites (1-1-6)	4	S-4	9	32	19	19
	5	S-5	10	40	21	23
Quinine, 2.0 grams per day on a similar	1	S-6	10	36	11	11
1-1-6 schedule	2	S-7	10	38	12	13
	3	S-8	10	39	11	11
	4	S-9	10	38	12	12
	5	S-10	10	40	12	16
Controls: no drug	1	S-11	10	36	11	11
	2	S-12	10	38	12	12
	3	S-13	10	39	12	11
	4	S-14	10	40	12	11

* Subjects with corresponding set numbers (1 to 5) were bitten by the same mosquitoes.

† The infective inoculum for each volunteer is expressed as the sum of the individual sporozoite densities in the mosquitoes which bit the volunteer.

‡ Because of toxic reaction, pentaquine was discontinued 1½ days after infection.

ATTEMPTED PROPHYLAXIS WITH PENTAQUINE-QUININE

Jones *et al.* (1948) found that pentaquine, in dosage of 120 mgm. of base per day on the day before, the day of, and for six days after the bites of ten infected mosquitoes (i.e., a 1-1-6 schedule), protected four out of five volunteers against Chesson strain *vivax* malaria, whereas 180 mgm. per day on a 1-1-6, 1-1-3, or 1-1-2 schedule gave complete protection of all subjects. These dosages were all definitely toxic. It was considered of theoretical interest to determine whether sufficient potentiation occurred when quinine was given concurrently for complete prophylaxis to be achieved with smaller doses of pentaquine.

Experiment. Five volunteers (S-1 through S-5) were given pentaquine in dosage of 60 mgm. of base per day (15 mgm. every 6 hours) and quinine in dosage of 2 gm. of base per day (0.5 gm. every 6 hours) for one day before, on the day of, and for six

TABLE 2

Comparative relapse data in volunteers treated at each attack with pentaquine-quinine, pentaquine-cinchonidine, pentaquine-cinchonine or quinine

VOL. NO.	INOCULUM (SUM OF PLUSES)	TOTAL NO. OF ATTACKS	NUMBER OF DAYS FROM END OF TREATMENT TO RELAPSE BETWEEN ATTACKS. (— INDICATES NO RELAPSE)												DAY OF LAST PARASITEMIA*
			1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	13–14	
Treatment with pentaquine and quinine																
S-1†	36	1	—	—	—	—	—	—	—	—	—	—	—	—	—	26
S-5†	40	1	—	—	—	—	—	—	—	—	—	—	—	—	—	29
S-9	38	2	59	—	—	—	—	—	—	—	—	—	—	—	—	90
S-13	39	1	—	—	—	—	—	—	—	—	—	—	—	—	—	17
S-39	37	1	—	—	—	—	—	—	—	—	—	—	—	—	—	15
S-40	38	2	15	—	—	—	—	—	—	—	—	—	—	—	—	47
Treatment with pentaquine and cinchonidine																
S-2†	38	1	—	—	—	—	—	—	—	—	—	—	—	—	—	22
S-6	36	7	6	11	15	18	57	72	—	—	—	—	—	—	—	304
S-10	40	2	40	—	—	—	—	—	—	—	—	—	—	—	—	77
S-14	40	3	10	14	—	—	—	—	—	—	—	—	—	—	—	77
S-25	35	2	52	—	—	—	—	—	—	—	—	—	—	—	—	86
S-38	38	2	13	—	—	—	—	—	—	—	—	—	—	—	—	47
Treatment with pentaquine and cinchonine																
S-4†	32	1	—	—	—	—	—	—	—	—	—	—	—	—	—	24
S-8	39	3	10	41	—	—	—	—	—	—	—	—	—	—	—	101
S-12	38	2	9	—	—	—	—	—	—	—	—	—	—	—	—	42
S-23	40	3	12	124	—	—	—	—	—	—	—	—	—	—	—	186
S-26	37	3	13	48	—‡	—	—	—	—	—	—	—	—	—	—	105
S-37	40	2	43	—‡	—	—	—	—	—	—	—	—	—	—	—	78
Treatment with quinine																
S-3†	39	8	12	16	28	26	26	36	40	—	—	—	—	—	—	325
S-7	38	11	7	10	10	11	14	16	16	30	103	20	—	—	—	432
S-11	36	13	6	7	8	8	10	13	15	16	18	29	56	48	—	461
S-24	38	11	6	8	11	12	13	16	34	49	62	100	§			519
S-41	40	13	8	8	10	10	11	15	19	24	32	36	25	44	—	470

* All men were under observation for at least 540 days except S-26 (359 days) and S-37 (144 days).

† Prior course of protective 8-aminoquinoline therapy.

‡ Parole or transfer without followups.

§ 11th attack left untreated with intermittent parasitemia for 19 days.

days after exposure. Five men were given the same dosage of quinine without pentaquine and four men served as controls (Table 1).

On 12 July 1946 each of the 14 men was bitten by ten heavily infected mosquitoes.

Volunteer S-3 had a severe hemolytic reaction which necessitated discontinuance of pentaquine after the first dose on the fourth day of administration, so that his actual regimen was 1-1-1$\frac{1}{4}$. The details of this episode will be presented subsequently.

All of the men experienced attacks of malaria, as shown in Table 1. In those who received full doses of pentaquine and quinine, patent parasitemia did not appear until 15, 19, 21 and 21 days after exposure, respectively. All other volunteers in the experiment developed patent parasitemia 11 to 12 days after the bites.

After development of acute attacks, subjects S-1 and S-5 were treated with pentaquine (60 mgm. per day) plus quinine (2 gm. per day) for 14 days; S-2 was given pentaquine and cinchonidine for the same period; S-3 was given quinine alone; S-4 was given pentaquine and cinchonine. In each case the relapse pattern appeared to be modified by the protective course of pentaquine-quinine. (See Table 2). Patients in the latter group were the only ones we have observed who were cured by single courses of pentaquine-cinchonidine or pentaquine-cinchonine.

Comment. A regimen consisting of 60 mgm. of pentaquine and 2 gm. of quinine continued for six days after exposure did not prevent infection with the Chesson strain of *P. vivax*. The delay in appearance of the infections (even though both pentaquine and quinine are quickly eliminated from the body) and the fact that the infections were more easily cured than were those in the controls, suggest that the regimen was partially prophylactic. It will be recalled that Jones *et al.* (1948) found that prophylactic regimens of 8-amino-quinoline drugs often reduced the likelihood of repeated relapses, presumably by decreasing the reservoir of exo-erythrocytic parasites.

STUDIES WITH PENTAQUINE-CINCHONIDINE AND PENTAQUINE-CINCHONINE

Alving *et al.* (1948) presented evidence to show that concurrently administered quinine enhanced the curative effect of pentaquine. The following experiment was carried out to determine whether or not cinchonidine or cinchonine could be substituted for quinine in this synergistic relationship.

Experiment. Twenty-three volunteers were used for the trials (Table 2). Successive attacks in each man were treated with the same combination of drugs. The daily amounts were divided into four equal doses, six hours apart, and continued for 14 days. Regimens were as follows:

a. Pentaquine, 60 mgm. per day plus quinine, 2 gm. per day
b. Pentaquine, 60 mgm. per day plus cinchonidine, 2 gm. per day
c. Pentaquine, 60 mgm. per day plus cinchonine, 2 gm. per day
d. Quinine, 2 gm. per day

Neither pentaquine-cinchonidine nor pentaquine-cinchonine prevented relapses as well as did pentaquine-quinine. The men given the less effective combinations, however, had shorter courses with more erratic and widely-spaced relapses, than the men given quinine alone. The latter continued to experience relapses for 15 to 17 months (Figure 1). As previously noted, volunteers S-1 through S-5, who had received prior dosage with pentaquine-quinine, showed a lower relapse potential than any of the other men treated according to the above regimens.

Plasma concentrations of pentaquine and of the cinchona alkaloids are shown in Table 3.

Comment. Cinchonidine and cinchonine apparently cannot be substituted for quinine on an equal dosage basis in pentaquine-quinine therapy. In a group of Ches-

son strain infections of more than average severity, the use of the above alkaloids with pentaquine resulted in a marked shortening of most infections, however, sug-

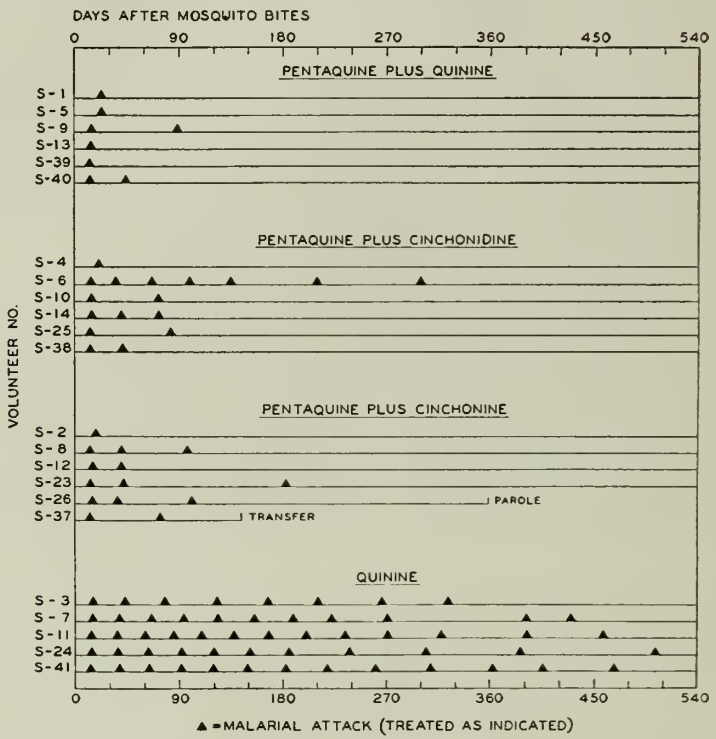

FIG. 1 Diagram of malarial attacks in 23 volunteers with Chesson strain vivax malaria.

TABLE 3

Plasma concentrations of pentaquine and of the cinchona alkaloids in volunteers treated with three different regimens. Dosage in all cases was 60 mgm. of pentaquine plus 2 grams of cinchona alkaloid per day for 14 days

REGIMEN	NO. OF COURSES	PLASMA CONCENTRATIONS			
		Pentaquine (μg. per liter)		Cinchona alkaloid (mg. per liter)	
		Range of means	Mean	Range of means	Mean
Pentaquine-quinine	6	24–133	73	7.8 –13.8	10.6
Pentaquine-cinchonidine	16	48–230	111	1.4 – 4.1	2.8
Pentaquine-cinchonine	12*	52–216	139	0.34– 1.8	0.8

* Abnormally high mean of 662 during first course of treatment in Volunteer S-26 excluded from calculations.

gesting a partial potentiation. Since there were no parallel controls treated with pentaquine alone, the actual contribution made by the cinchonidine and cinchonine cannot be clearly defined.

Taggart *et al.* (1948), in carefully conducted studies of the metabolism and antimalarial action of the various cinchona alkaloids, found that on an oral dosage basis cinchonine and cinchonidine were approximately one-half as active as quinine against the erythrocytic forms of *vivax* and *falciparum* malaria. Concurrent studies on plasma concentrations led them to believe that it was the rapid metabolism and elimination of those alkaloids that reduced their antimalarial efficiency, and that in terms of actual blood concentrations they were more active than quinine. The same factors are probably involved in reducing their potentiating action.

The small number of cases makes it impossible to reach final conclusions as to significance of the higher levels of pentaquine in blood plasma when cinchonine and cinchonidine were given concurrently. There was no concomitant increase of toxic reactions.

VARIOUS DOSAGE COMBINATIONS OF PENTAQUINE AND QUININE

In the course of experiments at Seagoville, pentaquine and quinine have been used in many other cases for the attempted cure of Chesson vivax infections. The results of certain of these trials will be presented because they shed light upon the difficulties encountered in attempting to establish minimal dosages for radical cure.

Experiments. In the summer of 1947 five volunteers (S-84 through S-88), who had been infected on 22 May 1947 by the bites of ten moderately infected mosquitoes, were treated with pentaquine and quinine at the time of their third attacks. Each of these men had had two earlier attacks terminated by intramuscularly administered chloroquine (Culwell *et al.*, 1949). At the time of our experiment the findings of Coggeshall and Rice (1949) that daily dosage with 30 mgm. of pentaquine and 1.0 gm. of quinine had proven curative in late relapsing *vivax* infections, were known to us, and this regimen was chosen for our group of five men. All five men, however, experienced relapses within 12 to 13 days. Two were cured after a second course, two required three courses and one required four courses of the stated dosage.

It was then decided to treat other subjects, infected by the same lot of mosquitoes and who had relapses following other noncurative therapy, with larger doses of pentaquine and quinine. Three men (S-97 through S-99, who were bitten by the same mosquitoes that fed on S-85, S-86 and S-87, respectively) were given pentaquine 60 mgm. per day, and quinine 2 gm. per day, at the time of their fourth attacks, which occurred approximately 14 weeks after mosquito bites. All three men experienced relapses 26 to 105 days after treatment; one required three courses of this dosage before cure. This can be contrasted with the results in the small series of 12 July 1946 (Table 4).

In a subsequent experiment, begun on 13 February 1948, 16 volunteers (S-144 through S-159) were used in an attempt to determine the minimal amount of quinine required to achieve curative potentiation when given with 60 mgm. of pentaquine per day for 14 days. The dosages and results are shown in Table 4. In all pentaquine-quinine regimens at least one-half of the infections were cured by one course, and even the quinine-treated subjects did not have a pattern of relapses comparable to that seen in many earlier subjects similarly treated. Their relapses were sporadic, even though their total courses of infection were as long as we had observed in volunteers such as S-7, S-11, S-24, S-41 (Table 4).

Comment. These results illustrate the variability in the amounts of pentaquine and quinine that may be needed for the cure of *vivax* malaria in different groups of patients. All of the volunteers included in this presentation were infected by the bites of ten infected mosquitoes, the sporozoite densities in the mosquitoes' salivary glands

TABLE 4

Comparative relapse data in volunteers treated with various regimens of pentaquine and quinine

VOL. NO.	DATE OF INOCULATION	SUM OF PLUSES	DOSAGE IN GRAMS OF BASE PER DAY FOR 14 DAYS		TOTAL NO. ATTACKS*	PARASITE-FREE INTERVALS FROM TREATMENT TO RELAPSE BETWEEN ATTACKS											DAY OF LAST PARASITEMIA
			Penta-quine	Quinine		1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–10	10–11	11–12	12–13	
S-84	22 May 47	31	0.03	1.0	3	†	†	12	18	—	—	—	—	—	—	—	—	162
S-85	"	29	0.03	1.0	2	†	†	12	—	—	—	—	—	—	—	—	—	94
S-86	"	26	0.03	1.0	2	†	†	12	—	—	—	—	—	—	—	—	—	116
S-87	"	28	0.03	1.0	4	†	†	12	23	54	—	—	—	—	—	—	—	225
S-88	"	19	0.03	1.0	3	†	†	13	110	—	—	—	—	—	—	—	—	237
S-9	12 Jul. 46	38	0.06	2.0	2	59	—	—	—	—	—	—	—	—	—	—	—	90
S-13	"	39	0.06	2.0	1	—	—	—	—	—	—	—	—	—	—	—	—	17
S-39	2 Sept. 46	37	0.06	2.0	1	—	—	—	—	—	—	—	—	—	—	—	—	15
S-40	"	38	0.06	2.0	2	15	—	—	—	—	—	—	—	—	—	—	—	47
S-97	22 May 47	30	0.06	2.0	2	†	†	†[1]	26	—	—	—	—	—	—	—	—	143
S-98	"	26	0.06	2.0	3	†	†	†	57	305	—	—	—	—	—	—	—	492
S-99	"	26	0.06	2.0	2	†	†	†	105	—	—	—	—	—	—	—	—	226
S-144	13 Feb. 48	32	0.06	2.0	1	—	—	—	—	—	—	—	—	—	—	—	—	72
S-155	"	32	0.06	2.0	2	346	—	—	—	—	—	—	—	—	—	—	—	376
S-145	"	32	0.06	1.0	1	—	—	—	—	—	—	—	—	—	—	—	—	74
S-150	"	37	0.06	1.0	2	125	—	—	—	—	—	—	—	—	—	—	—	197
S-152	"	30	0.06	1.0	1	—	—	—	—	—	—	—	—	—	—	—	—	32
S-156	"	36	0.06	1.0	1	—	—	—	—	—	—	—	—	—	—	—	—	17
S-146	"	32	0.06	0.5	1	—	—	—	—	—	—	—	—	—	—	—	—	80
S-151	"	32	0.06	0.5	5	14	42	15	65	—	—	—	—	—	—	—	—	216
S-153	"	36	0.06	0.5	1	—	—	—	—	—	—	—	—	—	—	—	—	38
S-157	"	37	0.06	0.5	1	—	—	—	—	—	—	—	—	—	—	—	—	19
S-147	"	37	0.06	—	2	103	—	—	—	—	—	—	—	—	—	—	—	163
S-149	"	32	0.06	—	1	—	—	—	—	—	—	—	—	—	—	—	—	18
S-154	"	36	0.06	—	6	9	9	9	22	13	—	—	—	—	—	—	—	210
S-158	"	32	0.06	—	1	—	—	—	—	—	—	—	—	—	—	—	—	20
S-7	12 July 46	38	—	2.0	11	7	10	10	11	14	16	16	30	103	20	—	—	432
S-11	"	36	—	2.0	13	6	7	8	8	10	13	15	16	18	29	56	48	461
S-24	1 Sept. 46	38	—	2.0	11	6	8	11	12	13	16	34	49	62	100	‡	—	519
S-41	2 Sept. 46	40	—	2.0	13	8	8	10	10	11	15	19	24	32	36	25	44	470
S-148	13 Feb. 48	35	—	2.0	6	36	107	17	69	174	—	—	—	—	—	—	—	527
S-159	"	30	—	2.0	4	8	20	397	—	—	—	—	—	—	—	—	—	492

* Refers only to attacks treated with pentaquine, quinine or both.

† These attacks treated with regimens other than pentaquine and quinine.

‡ Eleventh attack left untreated.

were all in an acceptable range, and prepatent periods in the patients were 10 to 14 days. Nevertheless, there were marked differences in response to curative therapy. Thus in our volunteers infected on 22 May 1947 even 60 mgm. of pentaquine plus 2 gm. of quinine failed to cure, whereas in the group infected 13 February 1948,

pentaquine without quinine cured two of four and even the quinine relapse patterns were substandard.

Alving *et al.* (1948) have experimental evidence that men infected by the bites of 60 to 80 mosquitoes are not cured by conventional doses of pentaquine and quinine. Our experience would indicate that even with a standard ten mosquito-inoculum the challenge may sometimes be overwhelming, even at the time of a third or fourth attack of malaria.

Comparisons of the curative abilities of drugs tested at different times must therefore be guarded, and parallel controls are essential for definitive comparisons.

TOXIC REACTIONS

Observations. Pentaquine has been used by us in 112 courses of treatment in 62 different volunteers. This includes several patients not included in other portions of the present paper.

In those given 30 mgm. of pentaquine per day (14 courses in 5 patients) there were no reactions except mild abdominal discomfort in five instances.

During 98 courses in which a dosage of 60 mgm. of pentaquine per day was given (with or without concurrent medication) a considerable number of adverse reactions were encountered. The most frequent, such as abdominal discomfort, nausea, vomiting, anorexia and cyanosis became commonplace to the volunteers and observers, so that they were not uniformly recorded. The result is that exact incidence data on these cannot be given. In no instance, however, did such complaints necessitate discontinuance of therapy.

Another frequent reaction was fever attributable to the drug. In 16 men temperature of above 101°F. was recorded during pentaquine therapy at times when it was unlikely or impossible for it to have been the result of malaria. For example, in four of ten men given pentaquine and quinine prophylactically or during latency, such fever occurred. Temperature elevations attributed to the drug were observed on all days of therapy from the 3rd to the 14th, but most instances were on the 6th, 7th and 8th days (4, 7 and 4 patients, respectively). Since it is impossible to blame the drug for fever during the first four days of treatment of febrile attacks of malaria, all instances of early fever we have reported occurred in patients who were not experiencing active malaria. Although drug was discontinued in two men with febrile reactions, this was because of other findings; in the other cases fever was self-limited even though the drug was continued.

Nine patients who experienced drug fever during their first course were given pentaquine again. None had fever again, although one man had two, one had four and one had six additional courses of drug.

Hemolytic reactions. During the 112 courses of therapy there were 15 instances in which hemoglobin concentrations dropped 10 per cent or more during the course of pentaquine therapy. Eight of these occurred on a regimen of 60 mgm. plus 2 gm. of quinine; one on a regimen of 60 mgm. plus 1 gm. of quinine; one on 60 mgm. of pentaquine alone; one on 60 mgm. plus cinchonidine and four on 60 mgm. plus cinchonine. Of these 15 instances, three were sufficiently dramatic to be considered as examples

of acute intravascular hemolysis; one of these was alarmingly severe. Case reports follow.

Volunteer S-3, a 26-year-old white Jewish male, was started on a regimen of pentaquine (60 mgm. per day) and quinine (2 gm. per day) on 11 July 1946, the day before exposure to the bites of ten infected mosquitoes. On the evening of 12 July his temperature rose to 100.2°F. with pulse of 108. On 13 July he complained of nausea and abdominal cramps and vomited several times. It was noted that his urine was deep amber and that he had a questionable icteric tinge to his skin. On the following morning, 14 July and the 4th day of drug administration, he was markedly jaundiced. Icterus index was found to be 114 with serum bilirubin 7.2 mg. per 100 ml. Both urine and blood plasma were dark in color. Demonstration of a drop in hemoglobin to 13.9 gm. per 100 ml. from an original 19.3 gm. and a drop in red blood cell count to 3.5 million from an original 5.7 million confirmed a diagnosis of intravascular hemolysis. Pentaquine administration was discontinued after the 13th dose, but quinine was continued. Fluids were given intravenously, but due to difficulties in obtaining blood which gave satisfactory cross-matching, blood transfusion could not be given until the following morning, 15 July. By this time hemoglobin concentration in the blood had dropped to 8.4 gm. and red blood cell count to 1.97 million. One thousand ml. of blood were given on 15 July and 500 ml. on 16 July. There was rapid improvement and by 21 July the volunteer had no complaints. This man experienced seven subsequent attacks of malaria without undue difficulty; he was never given pentaquine again.

Volunteer S-26 was the second man observed during a severe reaction. He was given pentaquine 60 mgm. per day plus cinchonine 2 gm. per day for treatment of a primary attack. Almost from the start of therapy he exhibited severe nausea and vomiting. Although fluid intake and urinary output were maintained by intravenous administration of saline and glucose solutions, the plasma concentrations of pentaquine rose rapidly. Twenty-four hours after the first dose it was 370 micrograms per liter. On the 4th day of administration, the plasma pentaquine concentration was 630 micrograms per liter. At this time hemoglobin was 14.5 gm. as compared with an initial 16.9 gm.; red blood cell count was 3.14 million as compared with an initial 5.21. The patient was frankly cyanotic, with 14.3 per cent of hemoglobin converted to methemoglobin. Urine was only slightly discolored and there was no clinical icterus. On the 7th day of drug administration hemoglobin had dropped to 11.9 gm. with a red blood cell count of 3.12 million. Urinary output continued to be satisfactory, but urine showed moderate albumin, occasional red blood cells and red blood cell and granular casts. Urobilinogen output was increased and the patient was faintly icteric with total serum bilirubin of 1.6 mgm. per 100 ml. Irregular fever which had continued after cessation of malaria went as high as 103.6°F. Drug was stopped on the 8th day, after it was found that the plasma concentration of pentaquine had reached 1,020 micrograms per liter. After discontinuance of drug there was rapid improvement. The plasma concentration of pentaquine was below detectable limits 72 hours after the last dose. Jaundice and methemoglobinemia gradually cleared. White blood cell count, which had been within normal limits during the foregoing reaction, dropped to 4,400 during convalescence, but there was no disturbance of the differential count.

This volunteer was given another course of the same drug combination only 15 days later and again plasma concentrations were higher than average, ranging from 150 to 450 micrograms per liter, but symptoms never became severe. A third course eight weeks after the second was well tolerated.

A third moderately severe hemolytic reaction, with somewhat different manifestations, was noted in volunteer S-145. This man, in his primary attack, was given 60 mgm. of pentaquine and one gram of quinine per day for 14 days. After the first dose he complained of transient wheezing and shortness of breath. On the 4th day he complained of substernal pain, nausea and extreme malaise. Despite clearance of malarial parasites from the peripheral blood, he continued to exhibit intermittent fever until the 11th day of medication, peaks being 103.6 on the 5th and 6th days, 103.4 on the 7th and 103 on the 8th days. Abdominal cramps, shortness of breath and substernal pain persisted. There was a gradual decline in hemoglobin from an initial 15.4 gm. per 100 ml. to 9.8 gm. per 100 ml. on the 11th day. On the latter day the methemoglobin percentage was approximately 6.8. There was no frank jaundice, but blood bilirubin rose to a maximum of 1.6 mg. per cent on the 12th day, at which time the icterus index was 25. Urine was normal except for a high urobilinogen (Ehrlich's benzaldehyde test was positive to 1:640 dilution on the 10th day and remained abnormally high until after the end of drugging). White blood cell count never went above 11,200 or below 5,600. Pentaquine concentrations in plasma ranged between 13 and 49 micrograms per liter, quinine concentrations between 4.0 and 8.3 milligrams per liter and methemoglobin between 4.3 and 11.7 per cent. This volunteer completed the full course of 14 days treatment. He had no further attacks of malaria and pentaquine therapy was not repeated.

Comment. In a series of 74 patients given pentaquine at a dosage of 60 mgm. per day, Alving *et al.* (1948) found none whose symptoms of toxicity warranted discontinuance of therapy. He found abdominal discomfort the leading symptom; anorexia and nausea were common. There were no instances of acute hemolytic anemia, but declines in hemoglobin occurred which could not be explained by malaria. The production of methemoglobin was common, but in only nine was cyanosis clinically evident. Fever on the 4th, 7th and 10th days was observed in three men.

In our series methemoglobinemia was more pronounced and there were more subjects with frank cyanosis. Fever was much more common. In addition, three men experienced intravascular hemolysis sufficient to produce mild to severe icterus and to reduce hemoglobin by 10.9 gm., 5.0 gm. and 5.6 gm. over periods of 4, 6 and 10 days, respectively. In the first two cases the drug was discontinued. Only in the first case was the hemolysis dramatic and alarming. In the second there was a striking elevation of pentaquine in the plasma. In the third case the reaction was milder, plasma concentrations remained in the normal range and improvement occurred in spite of continuance of drugging.

It is noteworthy that one of the men in the preceding group of three was able to tolerate two additional courses of pentaquine without serious reaction. Furthermore, the men who had experienced drug fever during their first course of pentaquine therapy did not have similar responses during subsequent courses.

Our experience with pentaquine confirms us in the general belief that pentaquine-

quinine should not be the treatment of choice for all cases of vivax malaria. The discomfort and potential risk are not justified except where re-exposure is unlikely and conditions point to the likelihood of repeated relapses. While abdominal pain, cyanosis and mild fever are not indications for discontinuance of therapy, the occasional occurrence of hemolysis is sufficient justification for close observation of patients and ready accessibility to well equipped medical facilities.

SUMMARY AND CONCLUSIONS

Pentaquine in dosage of 60 mgm. of base per day plus quinine 2 gm. of base per day, given for one day before, on the day of, and for six days after exposure to ten infected mosquitoes, did not prevent Chesson *vivax* malaria in any of five patients. There was evidence, however, that the combination was partially prophylactic in that it modified the subsequent course of the infections.

Neither cinchonine nor cinchonidine, when substituted for quinine in combined pentaquine-quinine therapy, resulted in comparably high cure rates of Chesson *vivax* malaria.

It was observed in the course of using pentaquine and quinine that patients infected by different lots of mosquitoes, even when the densities of sporozoites were in the same general range and the number of infective bites was the same, appeared to differ in their potential for relapse. In some series pentaquine 60 mg. plus quinine 2 gm. was curative, in others it failed completely even at the time of the third relapses. In the absence, therefore, of parallel controls extreme caution is required in drawing conclusions as to the relative curative activity of drugs.

During 112 courses of treatment with pentaquine, alone and in various combinations, there were many toxic reactions noted in addition to the expected incidence of abdominal discomfort, nausea, anorexia and cyanosis. Drug fever occurred in 16 men, being most noticeable on the 6th, 7th and 8th days of the first course of drug administration. Repetition of pentaquine therapy in the men who had previously experienced fever was uneventful, however. In 15 men, hemoglobin concentrations dropped ten per cent or more during the course of pentaquine therapy. In three instances there were profound reductions in hemoglobin, two of which were sufficient in conjunction with other findings to necessitate discontinuance of medication. One of these cases represented a severe acute intravascular hemolysis, the first that has been reported in a white subject.

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SUMARIO Y CONCLUSIONES

Pentaquina en dosis de 60 mgm. de base por día, más quinina a 2 gm. de base por día administradas diariamente desde la vispera y hasta los seís días subsiguientes a la exposición de picadas de 10 mosquitos infectados no previno la infección de la cepa Cheson de *vivax* en ninguno de cinco pacientes estudiados. Sin embargo, hubo evidencia de q. la combinación fué parcialmente profiláctica en cuanto que modificó el curso subsecuente de las infecciones.

Ni la cinconina ni la cinconidina usadas en lugar de la quinina en terapéutica combinada de pentaquina- quinina produjeron curación más rapida de la cepa Cheson de *vivax*.

En el curso de estos experimentos se observó que cuando se usa pentaquina y quinina, pacientes infectados con diferentes lotes de mosquitos aún cuando la densidad de esporozoitos y el número de picadas infectantes fueron iguales, parecieron diferir en cuanto a su tendencia a las recaídas. En algunas series 60 mgm. de pentaquina con 2 gm. de quinina fué una dosis curativa mientras que en otras falló completamente aún en el caso de terceras recaídas. En consecuencia, cuando no existen controles paralelos debe tenerse extrema precaución para llegar a conclusiones en cuanto al poder relativo de curación entre las diversas drogas.—

En 112 casos de tratamiento con pentaquina sola o en diferentes combinaciones se notaron muchas reacciones tóxicas en adición a las esperadas de molestias abdominales, nauseas, anorexia y cianosis. Fiebre por drogas ocurrió en 16 indivíduos, siendo más notoria en el 6°, 7° y 8° día del primer período de administración de la droga. Una repetición de la terapéutica de pentaquina en los individuos que habían sufrido fiebre, no tuvo, sin embargo, consecuencias apreciables. En 15 indivíduos las concentraciones de hemoglobina bajaron diez por ciento y más durante el período de terapéutica con pentaquina. En tres ejemplos hubo profundas reducciones en la hemoglobina, dos de las cuales complicadas con otros síntomas hicieron necesaria la interrupción del medicamento. Uno de estos casos presentó una severa hemolisis intravascular aguda, la primera que ha sido reportada en un indivíduo blanco.

ACQUIRED RESISTANCE TO CHLORGUANIDE IN THE PIGEON STRAIN OF *PLASMODIUM RELICTUM* (GRASSI AND FELETTI, 1891)[1, 2]

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Resistance to chlorguanide was reported in 1947 by Williamson and Lourie and by Bishop and Birkett who fed non-curative doses of the drug for approximately four months to chicks subinoculated serially with *Plasmodium gallinaceum*. Thompson (1948) demonstrated acquired resistance to the same compound in *Plasmodium lophurae* in chicks following seven months of treatment with less than minimal effective doses administered orally. Studies by Seaton and Lourie (1949) established the fact that *Plasmodium vivax* may become chlorguanide-resistant as a result of more than 20 months subeffective drug therapy. Cooper *et al.* (1950) confirmed this work with the Chesson strain of *P. vivax*. The present investigation was undertaken to determine if chlorguanide resistance could be developed in another species of *Plasmodium*, *P. relictum*, and to obtain additional information on the nature of the acquired resistance.

MATERIALS AND METHODS

In this study the white Carneaux strain of the pigeon, *Columba livia*, was used as the host, and the 1P strain (Huff, Boyd, and Manwell, 1942) of *Plasmodium relictum* as the infective agent. Birds were infected by intravenous inoculation of parasitized blood taken in citrated saline (0.45 g. sodium chloride, 1.9 g. sodium citrate, and 100 cc. distilled water) at the height of parasitemia. Degrees of parasitemia were expressed either as a) parasites per field, i.e., the range of parasite counts made in 10 to 75 oil immersion fields, the number of fields examined varying inversely with the density of the parasites, or b) as parasites per 10,000 red blood cells, based on the numbers of parasites in 1000 to 5000 red blood cells on Giemsa-stained thin blood films. Chlorguanide (hydrochloride salt) was prepared in a 200 mg. per cent aqueous solution and was administered intravenously in the usual manner or into the crop by means of an esophageal tube.

Beck (1948), working in this laboratory, had noted that pigeons manifest toxic symptoms to this compound given perorally in doses of 15 mg. or more. To preclude such an eventuality in the later stages of this investigation, the drug was first administered intravenously starting on the day of parasite inoculation. The initial amount was 1 mg. (half the minimal effective dose according to observations in this

[1] Presented before the National Malaria Society, November 7, 1949, in Memphis, Tenn.

[2] This research was suggested by and was carried out under the direction of Dr. William B. Redmond in fulfillment of requirements for the degree of Master of Arts. His supervision is gratefully acknowledged. The work is part of a research program made possible by funds assigned to Dr. Redmond by the Dazian Foundation for Medical Research.

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laboratory) repeated on the next day. This procedure was followed with four more pigeons, gradually increasing the daily doses from 1 to 2 mg. Thereafter, the drug was not injected until parasites appeared in the peripheral blood. Treatment then consisted of two or three doses on consecutive days increasing from 2 to a maximum of 5 mg. per dose in another series of five birds. Due to the increasing difficulty of maintaining the strain, it became necessary at this point to reduce the amount of medication, and doses of 2.5 or 3 mg., according to the level of infection, were administered through five more passages.

Since the intravenous injection of chlorguanide did not appear to produce parasite resistance, the course of the experiments was altered and the drug was given perorally during the remainder of the study. Doses of 2 mg. were administered on the day of inoculation and for one to three days thereafter through the next eleven serial transfers.

The development of parasite resistance to chlorguanide was determined by injecting four 2 mg. doses of drug intravenously or perorally into birds infected with the experimental and normal strains of *P. relictum* and by comparing subsequent parasite levels.

RESULTS

In the attempts to produce a chlorguanide-resistant strain of *P. relictum* in the pigeon there was no consistent evidence of its development at the end of the first fifteen serial transfers in which the drug had been injected intravenously (see Table 1). In fact, there seemed to be some indication that the parasites were becoming overly sensitive rather than resistant to chlorguanide as it became difficult to maintain the infection even in untreated birds. However, after serial passages through three birds in which the drug was administered perorally, evidence of developing resistance began to be manifested. From that point on, the density of parasites was regularly as high or higher than in untreated infections even though quantities of chlorguanide were being exhibited which ordinarily reduced or extinguished the parasitemia in the normal strain. In the eighth passage bird treated perorally, the amount of drug was increased to four doses of 4 mg. each. The level of parasitemia in this host was only slightly below that of other birds which received only half as much drug. The infection was terminated more abruptly in this bird, however.

Table 2 shows the results of drug challenges against the experimental and normal strains, indicating that resistance to chlorguanide was produced as the consequence of non-curative treatment through 27 serial transfers of the organism. Even when tested against a total of 8 mg. of chlorguanide, the experimental strain developed degrees of parasitemia higher than are generally noted in normal infections; on the other hand, the parasite levels of the unmodified strain did not reach usual heights and were promptly suppressed by the same amount of drug. In challenging each strain, the drug was administered both perorally and intravenously without producing significant differences in parasite levels.

Nine pigeons carrying latent infections of the normal *P. relictum* strain were available. These birds were injected with parasites of the resistant strain, but in only two of the nine were parasites found as late as 48 hours after inoculation. These were

TABLE 1

Production of chlorguanide-resistant strain of P. relictum

INTRAVENOUS SERIES				PERORAL SERIES			
Serial Transfer Bird	Drug (mg.) Doses	Total	Parasites per field*	Serial Transfer Bird	Drug (mg.) Doses	Total	Parasites per field*
1st	1+1	2.0	1–5	16th from Intravenous Series			<1
2nd	1+2	3.0	5–15	1st	4+2	6.0	1–5
3rd	2+1.5	3.5	1–5	2nd	2+2	4.0	1–5
4th	2+1.5	3.5	5–15	3rd	2+2	4.0	5–15
5th	2+2	4.0	<1	4th	2+2	4.0	5–15
6th	1.5+2	3.5	5–15	5th	2+2+2+2+2+2	12.0	15–30
7th	2+2+2	6.0	1–5	6th	2+2+2+2	8.0	15–30
8th	2.5+2.5+2.5	7.5	1–5	7th	2+2+2+2	8.0	5–15
9th	3+3+3+3	12.0	15–30	8th	4+4+4+4	16.0	5–15
10th	3.5+5	8.5	5–15	9th	2+2+2	6.0	15–30
11th	3+2.5	5.5	<1	10th	2+2+2	6.0	30–50
12th	2.5+3+3	8.5	1–5	11th	2+2+2+2	8.0	30–50
13th	3	3.0	1–5				
14th	3+3	6.0	1–5				
15th	3+3	6.0	1–5				
16th	None	0.0	<1				

* At the height of infection.

TABLE 2

Challenge of experimental and normal strains of P. relictum *with four 2 mg. doses of chlorguanide*

BIRD NUMBER	PARASITES PER 10,000 RED BLOOD CELLS Day of infection							
	1	2	3	4	5	6	8	9
Experimental Strain								
Ps 148	✱	✱	670 ✱	1350 ✱	1600			220
Ps 149	✱	✱	830 ✱	1670 ✱	1530			1130
Ps 150	✱	✱	470 ✱	720 ✱	1330			50
Ps 151	*	*	1060*	3240*	3680			Dead
Ps 152	*	*	790*	1830*	900			70
Ps 153	*	*	960*	1280*	1040			—
Normal Strain								
Ps 141	✱	✱	10 ✱	2 ✱	2	—		
Ps 160	✱	✱	22 ✱	10 ✱	—		2	
Ps 161	✱	✱	56 ✱	24 ✱	4		—	
Ps 157	*	*	60*	54*	4		2	
Ps 158	*	*	34*	4*	2		—	
Ps 159	*	*	44*	10*	4		—	

✱—2 mg. dose of drug administered perorally.

*—2 mg. dose of drug administered intravenously.

so few in number as to be considered insignificant. Thus it appears from this test that the immunologic identity of the parent strain has been retained by the chlorguanide-resistant strain.

DISCUSSION

The mechanism of acquired chlorguanide fastness by malaria parasites is unknown. It seems probable that the 1P strain of *P. relictum* is a composite of biotypes which vary among other characteristics in their sensitivity to this compound inasmuch as non-curative amounts of it reduce without eliminating the parasitemia. Whether an abnormally insusceptible strain emerges from this congeries by mutation—as suggested for *P. gallinaceum* by Williamson and Lourie (1947)—or by natural selection can only be conjectured. It appears that the latter alternative is fully as compatible with the behavior of the resistant strain of *P. relictum* as observed in these experiments.

Although drug resistance did not become evident as the result of intravenous treatment with chlorguanide during fifteen serial passages, it cannot be concluded that this experience played no part in the ultimate development of the resistance. Other workers (Williamson, Bertram, and Lourie, 1947; Williamson and Lourie, 1947; Thompson, 1948) found that many months of oral administration of the drug were required for the refractoriness to manifest itself. Hawking and Perry (1948) concluded that the peroral route is the most effective one for the administration of chlorguanide, an observation which may account for the prompt appearance of insusceptibility following the shift from intravenous to peroral medication in this work. But until both methods have been tested concurrently in the production of chlorguanide fastness, it should not be concluded that this phenomenon is produced more readily by one than by the other.

CONCLUSIONS

The following conclusions seem justified from this study:

1) That it is possible to produce chlorguanide resistance in the pigeon-*P. relictum* host-parasite system by treatment with sub-curative amounts of the drug,

2) That the developed strain successfully resists challenge with normally therapeutic doses of the drug administered either perorally or intravenously, and

3) That the drug-resistant strain does not appear to be immunologically distinct from the parent strain of *P. relictum*.

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EFFECTS OF VARIOUS MODIFICATIONS OF A MASS STAINING PROCEDURE ON THE TRANSFER OF MALARIAL PARASITES BETWEEN BLOOD FILMS

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When blood films are stained together by a mass procedure similar to that described by Barber and Komp (1924), the possibility exists that blood elements may transfer from one slide to another (Brooke and Donaldson, 1948). If these transferred blood elements happen to contain malarial parasites, falsely positive diagnoses of malaria can result. An obvious solution to the problem would be the staining of the slides individually, but, from a practical standpoint, some type of mass staining procedure is far more expedient when large numbers of slides are involved, as in surveys or research studies. The present studies, therefore, have been directed toward testing various modifications of the original Barber and Komp procedure in an attempt to eliminate, or at least to reduce materially, this possible source of error in a valuable laboratory technique.

MATERIALS AND METHODS

Since many of the specific details of the experimental procedure have been described in the first report by the authors (op. cit.), they will not be repeated here. Only the materials and methods common to a number of the experiments are presented in this section. The individual procedural modifications which have been tested are described in the section dealing with experimental data.

Preparation of blood films. Thick films were prepared with the following: (a) avian blood from a pigeon; (b) parasitized blood obtained from paretic patients infected with either *Plasmodium vivax* or *P. falciparum*;[1] and (c) normal blood from persons with neither past histories nor present evidence of malaria. Hereafter, the avian and parasitized blood films will be referred to as *positive films*, the others as *normal films*. All films were prepared on new slides cleaned as recommended by Wilcox (1943), and were allowed to dry for 24 to 48 hours before they were stained.

Wrapping of packages of slides. The slides were wrapped by the Barber-Komp technique in packages of approximately 25 slides each. Within a package, the normal and positive slides were alternated in such a way that the films were either *opposing* or *non-opposing*. In the former arrangement each normal film directly faced a positive film, while in the latter all of the films faced in the same direction. Earlier studies (Brooke and Donaldson, 1948) demonstrated that a higher rate of transfer of blood elements occurred when the slides were wrapped with opposing films. This arrangement was used in many of the experiments reported here in order to afford a more critical test of modifications designed to reduce or eliminate the transfer.

Staining. Packages of slides were placed in glass containers just large enough to

[1] The authors are indebted to the Laboratory of Tropical Diseases, Subsection on Epidemiology, National Institutes of Health, Milledgeville, Ga., for supplying the parasitized blood.

accommodate the required number of packages for a given experiment. Giemsa stain diluted 1 to 50 with buffered water (pH 7.0–7.2) was poured into the containers and the films were allowed to stain for 45 minutes. The films then were rinsed in buffered water for three minutes and placed on paper towels to drain and dry. The procedures of removing the slides or the staining solution from the containers are involved in the various modifications of the techniques and will be described below with the experimental data.

Examination of blood films. In order to determine the degree of transfer, each normal film was examined until the entire film had been searched or until transferred elements (either malarial parasites or avian erythrocytic nuclei) had been found. Normal films stained along with parasitized blood films were examined with the oil immersion objective. Those stained along with avian blood films were examined with the 16 mm. objective to locate avian cells which then were checked with the oil immersion objective. In some of the experiments, other portions of the slides, particularly the line marking the surface of the staining solution, were examined for transferred elements.[2]

EXPERIMENTAL RESULTS

Experiments involving over 1,600 normal blood films were performed to test the effects of the following conditions on the transfer of blood from slide to slide during mass staining procedures: (a) agitation of the staining solution; (b) flushing of the staining solution from the container; (c) staining of packages of slides separately; (d) addition of a surface active agent to the staining solution; and (e) addition of varying concentrations of a surface active agent. Because experiments testing several of the above factors were carried on simultaneously, certain groups of slides served as controls in more than one experiment.

Effect of agitation of staining solution. As tested in the original study (Brooke and Donaldson, 1948), the staining procedure involved the pouring out of the Giemsa solution from the containers after the films had been stained. This causes considerable agitation of the solution which might contribute to the transfer of blood from one slide to another. It was desired to determine if lifting the slides from the staining solution, which appears to cause less agitation, might eliminate or reduce the transfer of blood.

Three experiments were performed to test this factor. In two, avian blood was utilized for the positive slides, and in one, parasitized blood (*P. vivax;* 21,000 parasites per cu. mm.). The films were non-opposing within each package and from two to four packages of slides were stained in each container. At the end of the staining period, the experimental slides were lifted from the staining solution while the control sets had the staining solution poured out of the container.

Results. Examination of the normal blood films revealed that lifting the slide pack-

[2] The authors greatly appreciate the technical assistance of the following individuals in the preparation and examination of the blood films: Mr. Paul Guptill, Mrs. Rosalind Sasser, Miss Barbara Pender, and Mrs. Amy Callas. The authors are particularly grateful for the assistance of Miss Aimee Wilcox who checked a number of the questionable malarial parasites found on the normal human films.

ages from the staining containers reduced but did not eliminate the transfer of blood (table 1). In the avian blood experiments, an average of 16.2 per cent of the films were contaminated when the slides were lifted out as compared with an average of 32.8 per cent when the stain was poured from the containers. In the experiment using parasitized blood, 6 per cent of the normal blood films were contaminated when the slides were lifted out and 10 per cent when the solution was poured from the containers.

Effect of flushing staining solution from container. During the earlier studies, it was noted that many of the detached blood elements apparently float to the surface of the staining solution, as evidenced by the frequent contamination of the slides along the stain line. In view of this fact, it seemed that the procedure of flushing the staining solution from the containers might be an effective way of preventing transfer between slides. Seven experiments were performed which tested the effect of flushing.

TABLE 1

Effect of agitation of staining solution on transfer of blood elements between films during mass staining procedures (All films non-opposing)

TYPE OF BLOOD	SLIDES LIFTED OUT OF STAIN			STAIN POURED OUT OF CONTAINER		
	No. normal films	No. normal films contaminated	Percentage contaminated	No. normal films	No. normal films contaminated	Percentage contaminated
Avian blood films	25	3	12.0	25	14	56.0
	49	9	18.4	48	10	20.8
Totals	74	12	16.2	73	24	32.8
Parasitized blood films[1]	50	3	6.0	50	5	10

[1] *P. vivax*, 21,700 parasites per cu. mm.

Two, employing avian blood, had the slides wrapped in packages with non-opposing films; and five, employing parasitized blood, were wrapped with opposing films. Blood containing either *P. vivax* or *P. falciparum* parasites was used and the parasitemia ranged from 1,230 to 21,700 parasites per cu. mm. of blood. After staining for 45 minutes, the diluted Giemsa stain was flushed out of one-half of the containers by the careful addition of large quantities of buffered water. The packages of slides in the remaining containers that served as controls were lifted from the staining solution.

Results. Flushing the staining solution from the containers apparently eliminated the transfer of avian erythrocytes (table 2). In contrast, the control slides that were lifted from the staining solution had an average of 16.2 per cent of the normal films contaminated with avian erythrocytes.

In the experiments involving parasitized blood, flushing considerably reduced but did not eliminate the transfer. Of the normal slides, an average of 29.6 per cent was contaminated when the stain was flushed out and an average of 48 per cent when the slides were lifted from the staining solution. Since opposing films show higher

rates of transfer, these tests with parasitized blood were more critical than those with avian blood, in which the slides were arranged in a non-opposing manner.

Effect of staining packages of slides separately. It was demonstrated in the previous study that transfer of blood can occur from one package to another when they are stained together (Brooke and Donaldson, 1948). The present experiments were designed to test to what extent intrapackage transfer occurs when packages are stained separately. Four experiments were performed, all utilizing parasitized blood. The patients supplying the blood had either *P. vivax* or *P. falciparum* with parasitemias ranging from 1,230 to 21,700 parasites per cu. mm. of blood. One-half of the tests were performed with opposing films and one-half with non-opposing films. Each package consisted of 25 slides of which either 12 or 13 were normal films. In each

TABLE 2

Effect of flushing stain from container on the transfer of blood elements between slides during mass staining procedures

	STAIN FLUSHED OUT OF CONTAINER			SLIDES LIFTED OUT OF STAIN			SPECIES OF MALARIA AND PARASITEMIA (NO. PARASITES PER CU. MM. OF BLOOD)
	No. normal films	No. normal films contaminated	Percentage contaminated	No. normal films	No. normal films contaminated	Percentage contaminated	
Avian blood films—non-opposing	25	0	—	25	3	12.0	—
	50	0	—	49	9	18.4	—
	—	—	—	—	—	—	—
Totals	75	0	—	74	12	16.2	—
Parasitized blood films—opposing	25	0	—	25	5	20.0	Pv 1,230
	25	7	28.0	25	15	60.0	Pf 1,800
	25	11	44.0	25	12	48.0	Pf 9,600
	25	5	20.0	25	12	48.0	Pv 5,800
	25	14	56.0	25	16	64.0	Pv 21,700
	—	—	—	—	—	—	
Totals	125	37	29.6	125	60	48.0	

test, two packages were stained in separate containers and two packages were stained together, the latter serving as the controls. The staining solution was poured into into the containers, and after 45 minutes the packages of slides were lifted out and rinsed.

Results. In the eight packages which were stained separately, only 2 normal films, both in one package, were contaminated with parasitized blood (table 3). In the packages which were stained in pairs, from 2 to 15 normal films in each pair of packages were contaminated. As was to be expected, a higher rate of transfer occurred in those packages in which the opposing arrangement of films was used.

Effect of a surface active agent. As mentioned previously, it has been observed that many of the detached malarial parasites or avian blood cells rise to the surface of the Giemsa solution. Since the removal of this surface film by flushing did not eliminate the transfer of parasitized blood, it was desired to know what effect a surface active agent, by causing the detached blood elements to sink, might have on the rate

of transfer. Triton X-30[3] was selected as the surface active agent to be tested. This compound is readily soluble in cold water and is miscible with alcohol and glycerine. Being a non-ionic substance, it has no appreciable effect on the pH of solutions to which it is added.

Preliminary tests demonstrated that the addition of Triton X-30 did not interfere with the staining action of the Giemsa solution on thick films. In tests to determine the effect upon transfer of blood, it was decided to use 0.5 per cent Triton X30 in the diluted Giemsa solution. A preliminary report of the results has been published (Brooke and Donaldson, 1950). The following are the details of the experiments, together with additional data on the effect of the surface active agent.

Nine experiments were conducted to test the effect of 0.5 per cent Triton X-30. In all of the experiments the slides were wrapped with opposing films. Two of the

TABLE 3

Effect of staining packages separately on the transfer of blood elements between films during mass staining procedures

TYPE OF BLOOD AND ARRANGEMENT OF FILMS	PACKAGES STAINED SEPARATELY		PACKAGES STAINED TOGETHER		SPECIES OF MALARIA AND PARASITEMIA (NO. PARASITES PER CU. MM. OF BLOOD)
	No. normal films	No. normal films contaminated	No. normal films	No. normal films contaminated	
Parasitized blood films—non-opposing	25	2	25	4	Pf 9,600
	25	0	25	2	Pf 21,700
Parasitized blood films—opposing	25	0	25	5	Pv 1,230
	25	0	25	15	Pf 1,800
Totals	100	2	100	26	

experiments utilized avian blood and the other seven, parasitized blood containing either *P. vivax* or *P. falciparum* parasites. The parasitemias ranged from 1,230 to 63,300 parasites per cu. mm. of blood. Two staining procedures were tested: one involved lifting the slides from the staining containers and the other, pouring the stain out of the containers.

Results. Practically no difference in the transfer rate was noted with the two types of staining procedures, i.e., whether the stain was poured out or the slides lifted out. Therefore, the two staining procedures will not be designated in the following statement of the results.

In the experiments involving avian blood, the addition of the surface active agent reduced the average rate of transfer from 79 per cent in the control slides to 6 per cent in the experimental slides. In the experiments involving parasitized blood, al-

[3] Triton X-30 is a 33 percent aqueous solution of an alkalated aryl poly-ether alcohol and is a product of Rohm and Haas Company, Philadelphia, Pa. The trade name is carried as a means of identifying the product under discussion and does not necessarily represent endorsement of the product by the Public Health Service.

TABLE 4

Effect of 0.5% Triton X-30 added to the diluted Giemsa on the transfer of blood elements between films during mass staining procedures (Films all opposing)

	0.5% TRITON—GIEMSA			GIEMSA			SPECIES OF MALARIA AND PARASITEMIA (NO. PARASITES PER CU. MM. OF BLOOD)
	No. normal films	No. normal films contaminated	Percentage contaminated	No. normal films	No. normal films contaminated	Percentage contaminated	
Avian blood	50	4	8	50	39	78	
	50	2	4	50	40	80	
	—	—	—	—	—	—	
Totals	100	6	6	100	79	79	
	25	0	—	25	5	20	Pv 1,230
	25	0	—	25	15	60	Pf 1,800
Parasitized blood	50	0	—	25	12	48	Pf 9,600
	25	0	—	25	12	48	Pv 5,800
	50	0	—	25	16	64	Pv 21,700
	25	0	—	25	12	48	Pf 63,360
	25	0	—	25	6	24	Pv 7,500
	—	—	—	—	—	—	
Totals	225	0	—	175	78	44.6	

TABLE 5

Effect of varying concentrations of Triton X-30 in diluted Giemsa stain on the transfer of avian blood elements between films during mass staining procedures (Films all opposing.)

CONCENTRATION OF TRITON X-30 IN STAINING SOLUTION	NO. OF NORMAL FILMS	NO. OF NORMAL FILMS CONTAMINATED	PERCENTAGE NORMAL FILMS CONTAMINATED
Controls	50	37	74
	50	39	78
0.01	50	37	74
	50	34	68
0.05	50	13	26
	50	12	24
0.01	50	1	2
	50	7	14
0.25	50	3	6
	50	6	12
0.5	50	1	2
	50	1	2

though an average of 44.6 per cent of the normal films were contaminated when stained with ordinary Giemsa, no evidence of transfer could be found when the staining solution contained Triton X-30 (table 4).

Effect of adding varying concentrations of a surface active agent. Following the dis-

covery that 0.5 per cent Triton X-30 in diluted Giemsa markedly reduced or eliminated the transfer of blood elements, two experiments were performed to determine the minimum concentration of the surface active agent which would accomplish the same effect. Fifty positive films prepared from avian blood were stained along with fifty normal films to test the effect of each concentration in the two experiments. The opposing arrangement of films was used throughout. Five concentrations of Triton X-30 in diluted Giemsa stain were tested: 0.5, 0.25, 0.1, 0.05, and 0.01 per cent. Control slides were stained with ordinary Giemsa solution. After staining, the packages of slides were lifted from the containers, rinsed, and allowed to air-dry. Surface tension measurements[4] were made on each of the staining solutions.

Results. The thick films, the stain line, and other areas of the normal slides were searched for the presence of transferred avian erythrocytes. In general, the greater the concentration of Triton X-30 in the staining solution, the fewer normal slides were contaminated (table 5). The lowest concentration (0.01 per cent) of Triton X-30 caused relatively little reduction in the rate of transfer which was found in the control slides. However, some effect was apparent in that the films were noticeably cleaner than the controls and fewer slides were contaminated in areas outside of the films. The next higher concentration (0.05 per cent) reduced the contamination by two-thirds, and increasingly higher concentrations yielded still more reduction. As was true in previous experiments, however, the highest concentration (0.5 per cent) did not completely prevent transfer of the avian cells. It should be pointed out that these films were arranged in the opposing manner which favors transfer. The surface tension determinations demonstrated that 0.05 per cent concentration of Triton X-30 reduced the surface tension from 58.8 dynes per cm. to 30.2 dynes per cm. Higher concentration did not further reduce the surface tension to any marked extent.

DISCUSSION

It is evident from the results of the experiments reported herein that variations in the procedure of staining blood films in bulk will affect markedly the amount of transfer of blood elements from one slide to another. The transfer of blood between slides was reduced both by staining of packages of slides separately and by insuring less agitation of the staining solution. In these experiments, flushing the staining solution from the container seemed to be quite effective in reducing transfer. The use of a surface active agent, Triton X-30, appeared to eliminate the transfer of parasitized blood and materially reduced the transfer of avian blood elements. This effect of the Triton X-30 may result from its reduction of surface tension, thus permitting the detached blood elements to sink in the staining solution, and from its wetting action, which may make these elements less apt to adhere to slides and films.

Triton X-30 has no harmful effect on the staining actions of the Giemsa solution on thick films. Varying dilutions, ranging from 0.01 per cent to 0.5 per cent, of the Triton X-30 in Giemsa have been tested. Even with the greatest concentration, the parasites stained well. Grossly, the slides stained with the Giemsa-Triton X-30

[4] The surface tension determinations were made with the de Nuoy tensiometer by Dr. George T. Lewis of Emory University, Atlanta, Ga.

solutions appear slightly paler and pinker than those stained with the standard Giemsa. This may be due to the cleaner preparations that are obtained when Triton X-30 is added. On these slides the films show practically no precipitated stain, bacteria, or any other debris that may be found on stained films. The surface active agent that is effective in preventing transfer of blood apparently also prevents the adherence of other foreign particles to the slides.

The experiments with varying concentrations of Triton X-30 demonstrated that, in general, the greater the concentration of the surface active agent, the greater was the reduction in the transfer of blood. Therefore, even though the surface tension of the staining solution was not reduced markedly by increased amounts of the reagent above 0.05 per cent, it would probably be advisable to employ the greatest concentration tested, i.e., 0.5 per cent. Higher concentrations have not been tested since, apparently, 0.5 per cent is capable of preventing the transfer of malarial parasites.

Triton X-30 is both readily available and inexpensive. Undoubtedly, there are other surface active agents which would have a similar action in preventing the transfer during mass staining procedures. However, before using some other agent it would be advisable to test its effect on the staining action of Giemsa and to confirm its ability to reduce transfer. Experiments testing the transfer can be conducted fairly easily with avian blood, since the examination of the normal films can be accomplished rapidly with the 16 mm. objective.

Mass staining procedures such as the one advocated by Barber and Komp are invaluable in laboratories that stain large numbers of blood films for malaria. As was pointed out in an earlier paper, if it were necessary to stain slides individually in order to prevent transfer of parasites, the conduction of surveys for malaria would be greatly hampered. The authors feel that the simple modification of adding a surface active agent to Giemsa solution makes it possible to use mass staining procedures with greater confidence.

SUMMARY

An investigation of various modifications of a mass staining procedure was made in an attempt to prevent the transfer of malarial parasites. It was found that the transfer could be reduced by lessening agitation of the staining solution, by flushing out the staining solution from the containers, and by staining the packages of slides separately. However, in this study, transfer of malarial parasites was eliminated only by the addition of small amounts of a surface active agent (Triton X-30) to the Giemsa solution. This simple modification of the standard Giemsa technique not only makes it possible to use mass staining procedures with greater confidence but results in cleaner slides that are more easily examined for malarial parasites.

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SUMARIO

Se efectuó una investigación de diversas modificaciones de un procedimiento de coloración en masa de láminas en un intento de prevenir la transferencia de los parásitos maláricos. Se halló que la transferencia pudo ser reducida disminuyendo la agitación de la solución colorante, vacían dola de los recipientes y haciendo la coloración en paquetes separados de láminas. Sin embargo en este estudio la transferencia de parásitos maláricos pudo ser eliminada únicamente por la adición de pequeñas cantidades de un agente reductor de la tensión superficial (Triton X-30) a la solución Giemsa. Esta simple modificación de la técnica Standard de Giemsa no solamente hace posible el uso confiado de procedimientos de coloración en masa sino que produce láminas más limpias que facilitan el exámen de los parásitos maláricos.

BOOK REVIEW

Medical Parasitology. By William G. Sawitz, M.D. Blakiston Company, Philadelphia, June 14, 1950. 296 pp. $4.25.

According to the author this manual is intended as a Lecture and Laboratory Guide for students in their course in Medical Parasitology and was not written for parasitologists. The subject is presented from the medical rather than the zoological point of view to provide the information necessary for the understanding of parasitic diseases. The author has adhered closely to these objectives. The subject matter is presented in an abbreviated style. There are included ninety figures, of which about two-thirds have been copied. One color plate of malaria parasites is included. The drawings are schematic and the tabular presentation of the various stages of life cycles are very helpful. In the case of the arthropods more detail in the sketches would have been helpful.

In general the choice of material is good from the point of view of the medical student or physician. In the interest of brevity certain didactic statements are made which need qualification. The sections on diagnosis, life cycle and pathogenesis would seem adequate, but prevention would need expansion from the public health point of view. Treatment is covered in considerable detail and is modern. No reference to antibiotics in amebiasis is included. In certain places the spelling has been conventionalized, as for example, amebas and sporozoas.—E. Harold Hinman.

A METHOD OF INFECTING *AEDES AEGYPTI* WITH *PLASMODIUM GALLINACEUM* FROM CHICK EMBRYOS

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In 1945 Haas and Ewing described a method for infecting chick embryos with *Plasmodium gallinaceum* by hand-feeding one to three infected mosquitoes on each embryo. The technique here reported was developed when it later became necessary to reverse the line of transmission and to feed a large number of *Aedes aegypti* on a single infected embryo.

An 18- or 19-day old infected chick embryo is prepared by painting with iodine the air sac end of the egg shell. The shell over the air pocket is then broken with a blunt instrument and the shell and outer shell membrane are removed with sterile forceps to expose a major portion of the inner shell membrane lining the air sac.

The equipment used in the insectary is similar to that described by Johnson (1947) with one modification. Over the top of a glass lantern globe are attached two sheets of rubber dam. Each sheet has a centrally located slit one and one-half inches long and the two pieces of dam are oriented so that the openings are at right angles to each other. Into this globe are placed 100 six-day old *Aedes aegypti* which have been held without food for 24 hours. The egg containing the infected embryo, prepared as described above, is then gently forced partially through the openings in the rubber dam so that the mosquitoes contained in the globe have free access to the veins beneath the exposed shell membrane. A small amount of talcum powder sprinkled on the rubber dam facilitates forcing the embryo through the slits without damage. A goose-neck lamp containing a 60-watt electric bulb is placed a short distance above the egg to prevent excessive chilling of the embryo. Handled in this manner, the embryo will usually survive a two-hour exposure period in a room held at 75±3°F with a relative humidity of 70 to 75 per cent.

If the parasitemia is such that one would expect the embryo to live more than 24 hours and if it is desired to feed again at a later period, an initial exposure to mosquitoes outside the incubator in excess of one hour is not advised. In these cases the exposed membrane is covered with a sterile half egg shell and the embryo is returned to the incubator as soon as possible.

By this method it has been found that during an exposure period of one and a half hours 40 to 50 per cent of the mosquitoes will become engorged, with as many as 80 to 100 per cent of these subsequently showing sporozoites.

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A MORPHOLOGICAL ALTERATION IN *PLASMODIUM GALLINACEUM*

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For several years comparative studies have been made on the various substrains of *Plasmodium gallinaceum* maintained in this laboratory. In 1948 it was noted that the parasites of one substrain when passed from chick to chick embryos showed in the latter host a morphological characteristic which had not previously been observed.

MATERIALS AND METHODS

Figure 1 shows diagramatically the relationship between the several substrains or series of *P. gallinaceum* used in this study. An alternating chick—*Aedes aegypti* substrain, designated the S-P series, has been carried in this laboratory for several years (Haas, Wilcox, et al., 1948). From the 49th chick passage of this series a blood-passaged series, the A-S strain, was established in chicks and has been maintained continuously since then. On October 5, 1948 blood was withdrawn from a chick of the 23rd A-S passage and inoculated intravenously into embryos in order to establish a similar blood-passaged series in embryos; this was designated the A-S-E substrain It was in blood films made from embryos of the first passage of this newly established A-S-E series that parasites showing a distinct morphological change were first observed. For comparion, parasites of the I-V substrain were considered to be normal for blood-induced infections in the embryo. The I-V substrain is a blood-passaged embryo series which at that time had been maintained through 288 serial passages and the parasites of which were morphologically identical with those of the naturally transmitted type of infection as represented by the S-P series.

Approximately 50 per cent of the eggs used in this work were from Rhode Island Red chickens and the others, obtained from a local hatchery, were a mixture, principally from White Rocks, Barred Rock and New Hampshire breeds. The embryos were prepared and inoculated intravenously according to the techniques described by Haas, Feldman and Ewing (1945), with these exceptions: The blood smears are now made from the air pocket end of the embryo by painting that end of the shell with iodine, picking away the unoccupied part of the shell with sterile forceps, injuring a convenient blood vessel with sterile forceps and picking up the oozing blood with the corner of a sterile slide. Also in making the first dilution of blood for inoculation the citrated blood is centrifuged and the supernatant replaced with the same amount of normal saline. Normal saline is used instead of sodium citrate in making higher dilutions. We believe this change in technique together with the fact that embryos used are usually 14 or 15, instead of 11 days old, account for a very much lower death rate in embryos than found by Haas, Feldman and Ewing.

Examinations were made most often on the 5th or 6th day. Some were made on

the 4th day and a few on the 7th day. Not all embryos in each inoculated lot were examined microscopically, but samples from each lot were studied. A total of 2084 embryos was inoculated in the A-S-E strain alone. Of these, 1836 embryos lived the four days required for examination and microscopic examination was made on 319 embryos (17 per cent).

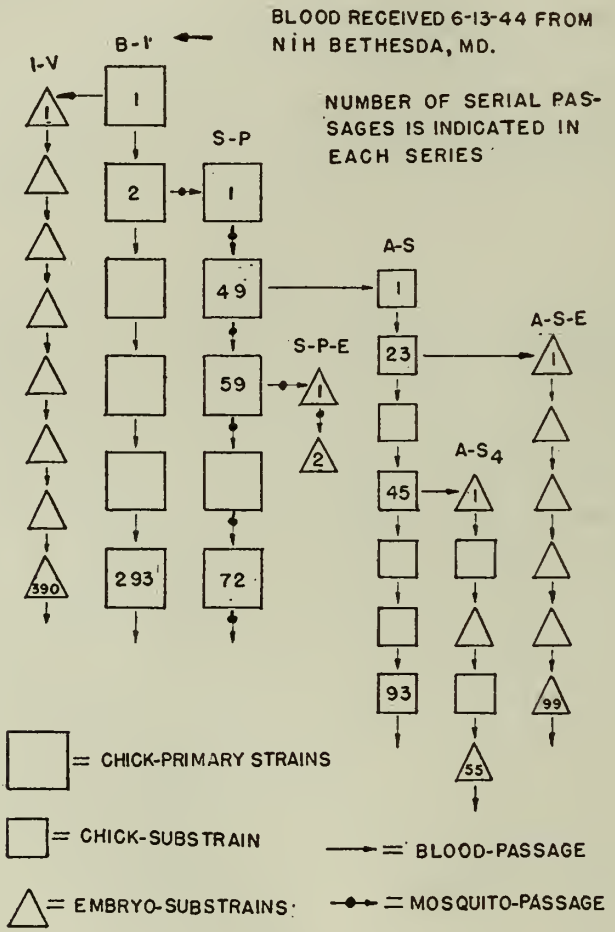

FIG. 1

RESULTS

In Giemsa-stained blood films from embryos of the A-S-E series many of the parasites showed large, clear, round, unstained areas with clean-cut edges (Fig. 2). For want of a better term these were called "vacuoles", although they were decidedly different from the irregular "food vacuoles" of growing parasites through which the color of the invaded cell can often be seen. These "vacuoles" were completely colorless. They were quite striking in appearance, particularly in the larger parasites. They were formed in the center of the parasite or at the edge and appeared as if a

rounded area had been punched out of the center or edge of the parasite with a keen, smooth-edged instrument. The pigment of the parasite was almost invariably associated with the "vacuole", either clumped or somewhat scattered in the clear

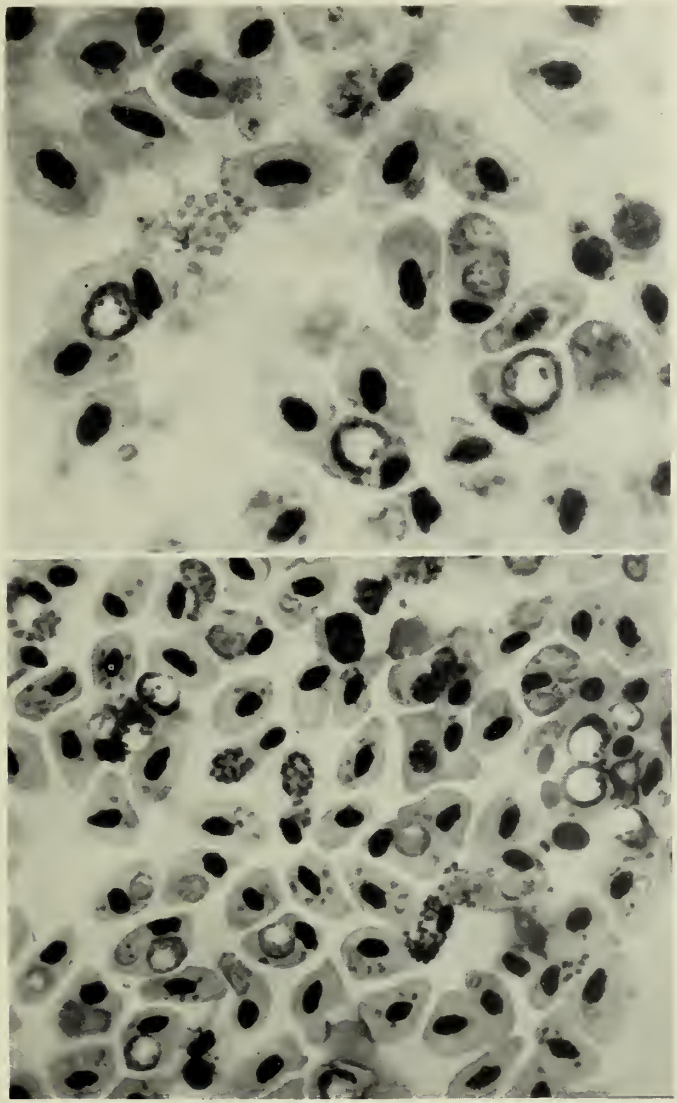

FIG. 2. Photomicrographs of embryo blood of A-S-E series showing the "vacuoles" in *P. gallinaceum* parasites. Upper photograph shows magnification of 1200 × and the lower one of 900 ×.

area, or strung in granules, like beads, along part of its edge. The parasites often seemed swollen and large for their respective ages and at times the parasitized cell was somewhat mis-shapen and slightly stretched in appearance. These "vacuoles" appeared in all stages of the parasite although they were largest and most noticeable

in the large trophozoites, the immature schizonts and the gametocytes. Embryos of successive passages in this A-S-E series continued to show "vacuolated" parasites.

It was impossible to ascertain by examination of unstained fresh blood whether the parasites contained "vacuoles" or not. Vaseline-ringed preparations of fresh blood on cover slips inverted on slides previously stained with brilliant cresyl blue were made for comparison with Giemsa stained slides of the same blood. The parasites in these preparations showed an equivalent number of areas that had the same clean-cut edges as the parasites in the stained slides. With the vital stain the vacuole-like areas took a solid, homogeneous, pale violet color, distinctly different from the rest of the parasite and cell, often extending out into the parasitized cell from the indented edge of the parasite as a violet disc. In or at the edge of the area was the pigment. It therefore appeared that the "vacuole" must be a part of the parasite that did not stain with Giemsa stain.

Attempts to stain the "vacuoles" with osmic acid fumes failed, even with 24-hour exposure. Therefore it may be presumed that they do not contain lipid.

In order to be certain that the phenomenon was not a peculiarity of this particular series, other series were begun. From the 56th chick passage of the S-P series, a blood series was carried in chicks for 11 passages and then put into embryos (A-S-E_2 series); and from the 57th chick passage of the S-P series, a third blood passage was carried in chicks for 5 passages and then put into embryos (A-S-E_3 series). The A-S-E_2 series was carried through 6 passages in embryos and the A-S-E_3 was carried 33 passages in embryos. Parasites containing "vacuoles" of the same type and size as found in the A-S-E series appeared in each of these series.

There was considerable variation in the number of parasites showing "vacuoles" in blood smears from embryos of the same or successive passages. Sometimes they appeared in abundance in a passage following one in which they were very scarce. Consequently it was necessary to carry the series for considerable time before the disappearance of the large "vacuoles" and the great reduction of the smaller ones could be definitely established.

The A-S-E series is now in its 100th passage. Large "vacuoles" ceased to appear in any noticeable number after the 43rd passage, and as the series has continued it has become indistinguishable in appearance from the regular I-V blood passaged series in embryos. (The I-V series came originally from a long line of chick blood passages and has at this time been carried through 390 transfers in embryos.) In the latter series a small "vacuole" may occasionally appear, a picture found also in the chick-mosquito series (S-P).

Transfer of blood from the embryo I-V strain into chicks for three serial passages and then back into embryos produced no "vacuoles" in the chicks and only a few small "vacuoles" in the embryo passage immediately following the chick passages. In the second passage there were considerably more small "vacuoles" (no large ones) while in the third embryo passage the parasites appeared normal. Likewise blood from the long carried chick blood-passaged B-I strain when put into embryos and carried for four serial passages produced a normal parasite picture. It therefore may be inferred that the long continued embryo blood-passaged strain has more or less lost its power to continue formation of these "vacuoles" even on alternation in the

chick and embryo hosts and that there may be some connection between the formation of large "vacuoles" in the parasites and a fairly recent passage of the parasite through mosquitoes.

Blood from the A-S-E series has been transferred directly to chicks in single passages on numerous occasions and the chicks have not shown "vacuoles" in their parasites. In addition to this, a series ($A\text{-}S_4$) of 55 transfers has been carried alternately through chicks and embryos by intravenous inoculations over a period of nearly 11 months. The blood for the first passage into the embryos came from the 45th passage of the A-S chick strain. All of the embryo passages and none of the chick passages have shown the "vacuoles" here discussed. Even though it is impossible to obtain vacuolated parasites in chicks by intravenous sub-inoculation, the chicks hatched from the A-S-E embryos always show "vacuoles" in their blood parasites. If mosquitoes fed upon these "vacuolated" parasites are then allowed to infect chicks, the resulting chick infections show no "vacuoles".

TABLE 1

Relation between Vacuole and Gametocyte Counts in Embryos of the A-S-E Substrain

PASSAGES	RANGE OF COUNTS IN 30 FIELDS		AVERAGE NUMBER IN 30 FIELDS		PER CENT NEGATIVE IN 30 FIELDS		PER CENT OF MOSQUITOES POSITIVE
	Vacuoles	Gametocytes	Vacuoles	Gametocytes	Vacuoles	Gametocytes	
1–33	0–283	0–152	63	35	1.6	3.3	85–100*
34–66	0–168	0–102	14	19	27	10	65–95**
67–99	0–29	0–67	6	4	58	16	0†

* Mosquitoes fed on six passages only.

** Mosquitoes fed on three passages only.

† Mosquitoes fed on one passage only.

The "vacuolated" parasites were used in one short mosquito-passaged series (S-P-E). Sporozoites from the regular chick-mosquito (S-P) series were inoculated into embryos both by mosquito feeding and by chorio-allantoic implant of sporozoites. The parasites were carried into a second lot of embryos by feeding of fresh mosquitoes on the infected embryos and by chorio-allantoic inoculation of sporozoites. Both lots of embryos showed "vacuoles" in the parasites, but chicks infected by mosquitoes from the same lots did not show "vacuoles" in the parasites.

It has been found repeatedly that "vacuolated" parasites can be obtained any time that a new blood-passaged series is started in embryos from a blood-passaged chick series recently established from the S-P series. Results identical with our experiences with the original series encourage us to believe that this work can be repeated at will.

Table 1 demonstrates the drop in the gametocyte count as the parasite was carried through repeated blood transfer, a tendency demonstrated previously in various species of malaria parasites. An interesting and possibly significant observation was the cessation of appearance of large "vacuoles" in the parasites coincident with the diminution in gametocyte numbers, although there was no direct correlation between number of gametocytes and number of "vacuoles" in individual passages. This table

is based on counts on 319 of the embryos that were inoculated over a period of 17 months. For comparison, counts on 25 passages of the I-V strain showed an average of 3 "vacuoles" and 6 gametocytes in 30 fields.

When the gametocyte rate was high, throughout the first third of the experiment, there was no difficulty in infecting mosquitoes and through them other chicks. Mosquitoes were infected on the 40th, 42nd and 43rd passages. In these passages 75 per cent of the mosquitoes examined had positive glands and 13 out of 15 chicks inoculated with the sampled lots of mosquitoes became positive. An attempt to infect mosquitoes was unsuccessful on the 97th passage, after the decrease in gametocytes and disappearance of large "vacuoles" had occurred.

Parasitemia has been consistently high in all A-S-E series throughout the 16-month period, ranging usually between 4000 and 9600 parasitized cells in 10,000 red blood cells on the 5th or 6th day, dependent upon the dilution of inoculum used. Exo-erythrocytic parasites have been found at autopsy throughout the study. Spleens show these forms more often than the other tissues although livers are almost as highly parasitized.

A paper by G. Gramiccia (1948) which came to our attention during the course of this study contains illustrations resembling closely the "vacuolated" parasites described above. In this and another paper (G. Rita and G. Gramiccia, 1947), a brief description is given of "vacuoles" found in the parasites of a small number of chicks hatched from eggs inoculated in various ways with blood containing *P. gallinaceum* parasites. The authors were unable to produce the "vacuolated" appearance by subinoculation into other chicks. No mention was made of finding the "vacuoles" in the unhatched embryo, or of carrying the "vacuolated" parasite by serial passage in embryos.

SUMMARY

1. A morphological alteration in *P. gallinaceum* is described.

2. This alteration consists of large, clear, round, unstained areas with clean cut edges occurring in many of the parasites. For want of a better term these areas are called "vacuoles".

3. These "vacuoles" persisted through many serial transfers in embryos by intravenous blood-passage. Eventually, the "vacuoles" became greatly reduced in size and number and finally a normal parasite picture was obtained.

4. Chicks inoculated with blood from these embryos show normal parasites. However, chicks, which hatch from embryos inoculated with the "vacuolated" strain, have "vacuoles" in their parasites.

5. Sporozoites from the mosquito-chick strain of *P. gallinaceum* when inoculated into embryos produce "vacuolated" parasites. However, chicks inoculated with sporozoites from the same lot of mosquitoes show a normal parasite picture.

6. Both gametocyte counts and "vacuole" counts decrease with continuous passage, large "vacuoles" in considerable number disappearing at about the same time that high gametocyte counts no longer appeared.

ACKNOWLEDGEMENTS

We are indebted to Miss M. Jean Vaughan, Mr. Harvey Akins, Mrs. Martha B. Austin, Misses Nell Coleman, Mary P. Coode and Carolyn J. Weaver for technical assistance in this work.

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SUMARIO

1. Se describe una alteración morfológica en *P. gallinaceum*.

2. Esta alteración consiste en grandes áreas, claras, redondeadas, no coloreadas y de márgenes claramente definidas que ocurren en muchos parásitos. A falta de un término más apropiado estas áreas se han llamado "vacuolas".

3. Estas vacuolas persisten a través de muchos pases en serie en embriones inoculados intravenosamente. Finalmente las vacuolas se reducen notablemente en tamaño y en número hasta que se obtiene un parásito normal.

4. Pollos inoculados con sangre de estos embriones muestran parásitos normales. Sin embargo, pollos que nacen de los embriones inoculados con cepa de "vacuola" tienen "vacuolas" en sus parásitos.

5. Esporozoítos provenientes de mosquitos con la cepa de *P. gallinaceum* cuando se inoculan en embriones producen parásitos con "vacuolas". Sin embargo, pollos inoculados con esporozoítos del mismo lote de mosquito muestran parásitos normales.

6. Las cuentas de gametocitos y de "vacuolas" disminuyen mediante pases contínuos. Las vacuolas grandes desaparecen en números considerables al mismo tiempo que bajan las cuentas de gametocitos.

THE LETHAL EFFECT OF THE CILIATE, *VORTICELLA MICROSTOMA* EHRENBERG ON *ANOPHELES QUADRIMACULATUS* LARVAE[1]

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At one time or another during the process of maintaining mosquito colonies, numerous investigators have experienced extensive mortality among the larval stages of certain species due to some disease agent which suddenly and unexpectedly contaminated the rearing pans. A number of protozoa and fungi in particular have been incriminated in the death of mosquito larvae and some of these have been shown to be definitely pathogenic (Steinhaus, 1949).

The writer has observed in two other laboratories sudden failure to rear *Anopheles quadrimaculatus* beyond the larval stage after a year or more of successful mosquito production. No attempt was made in either case to determine the pathogenic agent involved. It is the purpose of this note to report on a recent observation of a very similar nature in which the complete destruction of a colony of *A. quadrimaculatus* was caused by the peritrichous ciliate, *Vorticella microstoma*.

EXPERIMENTAL METHODS AND RESULTS

A colony of *A. quadrimaculatus* mosquitoes was established early this year from eggs sent through the courtesy of Dr. T. F. Hall of the Tennessee Valley Authority at Wilson Dam, Alabama. This and two additional colonies (*Aedes aegypti* and *Culex quinquefasciatus*) have been maintained continuously with no previous difficulty in rearing large numbers of all species. Approximately one month ago (June), however, the *Anopheles* larvae appeared to be dying and moved about the pans in a very sluggish, atypical fashion. Large numbers of dead larvae were noted on each of several subsequent days until none of the original 7,000 remained alive. The transfer of healthy-appearing ones to new pans with clean water did not prevent them from dying. The few that reached the pupal stage died shortly thereafter. Several of the living and dead larvae were then examined and each was covered with a thick, mossy-looking growth. Microscopically this was found to consist of an abundance of *Vorticella* with interspersed debris. The species was identified as *V. microstoma* with both attached and *telotroch* forms present.

Inasmuch as all species were reared in distilled water and received the same food, it was expected that the larvae of the other species would likewise be infested with these ciliates. Furthermore, the pipette used to pick pupae of all species was also used in removing dead *A. quadrimaculatus* larvae before the ciliates were discovered. Nevertheless, none of the *Aedes* or *Culex* larvae examined from each rearing pan were found to have attached *Vorticella*. Therefore it appeared that this ciliate exhibited a detrimental effect on the *A. quadrimaculatus* larvae only. In order to clarify

[1] The writer thanks Dr. Ludwik Anigstein for his enthusiastic interest in this study.

this point a series of experiments was carried out. The results are summarized in Table 1. It may be noted that all growth stages of *A. aegypti* and *C. quinquefasciatus*

TABLE 1

Resistance of culicine larvae to invasion by Vorticella

REARING PAN NO.	A. QUADRIMACULATUS* LARVAE		CULICINE LARVAE			MORTALITY RATE IN CULICINE LARVAE
	No.	Stage	No.	Stage	Species	
1	100	4th	1000	eggs	A. aegypti	0
2	200	"	500	1st	"	0
3	100	"	50	3rd	"	0
4	25	"	25	4th	"	0
5**	50	"	200	1st	C. quinquefasciatus	0
6	50	"	200	1st	"	0

* Dead larvae used entirely except in pan no. 2. All were covered with hundreds of living *Vorticella*.

** Pans no. 5 and 6 are duplicates. 100 additional *Vorticella*-infested *Anopheles* larvae added to each when the *Culex* larvae reached the 3rd stage.

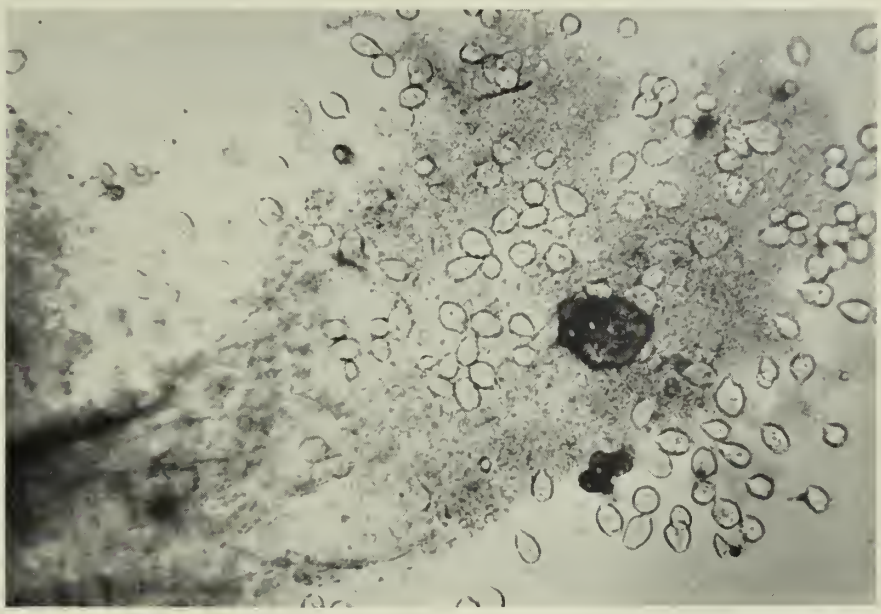

FIG. 1. *Vorticella* and associated debris adhering to small portion of 8th abdominal segment of 4th stage *A. quadrimaculatus* larva. (Photograph by F. W. Schmidt, Photographer, University of Texas, Medical Branch.)

used were uniformly refractory to invasion by *Vorticella* as judged by the lack of mortality in the culicine larvae when reared in pans with a great abundance of this ciliate. Moreover, daily examination of a number of these larvae from each pan revealed only an occasional one with a few attached ciliates. These did not interfere with the development in any way since all reached adulthood.

DISCUSSION

Jettmar (1947) in reviewing the literature on micro-organisms which attack mosquito larvae points out the divergance of opinion which exists in regard to the inimical effects of *Vorticella* on the larvae. Although some workers consider these ciliates to be harmless commensals, others look upon them as dangerous parasites which under certain circumstances may destroy an entire mosquito population. The same author observed a heavy *Vorticella* infestation in a large number of *Culex fatigans* larvae collected from a pond in Shanghai. While the first and second stages were almost completely free of the ciliates, the third and fourth stages were covered with them. This finding is in agreement with the present study in which no mortality was observed in the first two stages of *Anopheles* larvae. However, Jettmar found that the *C. fatigans* larvae had suffered a severe invasion by streptobacilli and that they were secondarily invaded by *Vorticella*. In the present study this ciliate appears to be the primary invader.

Although the exact mechanism whereby this peritrichous ciliate brought about death of the *Anopheles* larvae is not known, it appeared to be due to an interference with normal respiration. The majority of the larvae were almost completely covered with the growth and therefore seemed to have difficulty in locomotion and remaining on the water surface once it was reached. Furthermore, the intake of atmospheric air by the 8th abdominal spiracles was probably impeded by this growth. Figure 1 illustrates the density of attached ciliates and associated debris in this particular region of a 4th stage *A. quadrimaculatus* larva.

No explanation is advanced for the inability of the ciliates to infest the culicine larvae. The fact that these are reared in pans side by side with *Anopheles* larvae would suggest a specific selectivity on the part of the *Vorticella*.

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A STUDY TO EVALUATE MALARIA CONTROL PROJECTS OF KENTUCKY RESERVOIR IN TERMS OF COLLATERAL USES AND SOCIO-ECONOMIC BENEFITS[1]

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Throughout the history of the TVA malaria control program, conscious effort has been made to integrate malaria control with other TVA programs, activities, and objectives. In the design and construction of reservoir projects, full consideration was given to malaria control needs, which involved special water level management features in the dam structures, marginal clearing and drainage, malaria control equipment and facilities, major works to dewater or eliminate mosquito control problem areas, and purchase of malaria control easements to restrict to day-time occupancy certain marginal areas during malaria control season.

Because of the extent and the nature of malaria control works which have been established to provide effective and economical malaria control in the Kentucky Reservoir, there have been and are collateral opportunities for secondary uses which are consistent with the primary uses for malaria control and which contribute substantially to the over-all worth and benefit of the projects to the areas in which they are located. This is evident in view of the fact that permanent malaria control measures, such as diking and dewatering, filling and deepening of marginal flats, drainage of swamps in the margins of and adjacent to the reservoir, and development of malaria control grazing projects inevitably result in a certain degree of land reclamation or improvement for productive uses.

In addition to benefits directly related to productive land use, there are others, such as enhancement of recreation values as a result of effective mosquito control, elimination of unattractive mud flats at times of seasonal drawdown, public use of boat channels marked previously for malaria control purposes, and conversion of small boat operating bases to commercial recreation use when they are no longer needed for malaria control, the latter coming about as the result of the development of the use of DDT as a larvicide applied from airplanes. Even temporary emergency malaria control measures, such as house mosquito-proofing and DDT spraying of premises, which has superseded mosquito-proofing, contribute benefits other than malaria control through protection of families from flies and other insects, as well as malaria mosquitoes.

Gartrell and Kiker (1948) reported on the extent, direct value, and effectiveness of the various malaria control measures applied by TVA in the Kentucky Reservoir. Although the importance of collateral uses of malaria control works and benefits to other interests have been recognized, economic justifications of such projects have

[1] Information relating to this study was obtained from a number of sources, among which were the TVA Divisions of Reservoir Properties, Forestry Relations, and Construction and Maintenance, U. S. Fish and Wildlife Service, and the Tennessee Department of Conservation.

been based principally on malaria control requirements. However, savings in capital outlays for clearing, highway protection, road relocations, etc., were taken into account in establishing economic justifications where warranted and are a matter of record. Experience, particularly in the Kentucky Reservoir, has demonstrated diverse and large-scale use of malaria control projects for additional purposes.

The Kentucky Reservoir is the lowermost and largest of the main river reservoirs constructed by the Authority. The dam is located on the Tennessee River approximately twenty-two miles above its confluence with the Ohio. It backs water up to Pickwick Landing Dam, a distance of 184.3 miles. The lake at normal pool elevation, 359 feet, has an area of approximately 158,300 acres and a shoreline of 2,380 miles. Because of its size, the flat topography of the reservoir basin and some limitations on water level management, this project presents a malaria control problem of unusual magnitude and intensity.

Preliminary studies made prior to impoundment indicated that conventional malaria control measures would be exceedingly expensive and relatively ineffective for coping with the problem that was to be encountered in Kentucky Reservoir. During the preparation of the reservoir and in addition to the normal operations, such as reservoir clearance, marginal drainage, and staking of boat channels, the largest, most critical areas were studied for control by such methods as deepening and filling, diking and dewatering, and land-use restriction. As a result of this study and with the advice of a board of malaria consultants, TVA decided to deepen and fill eight large flat areas, dike for dewatering eight others, and apply the land-use restriction measure to one area.

Some of the uses and benefits resulting from these projects are discussed below by types of control measures:

DIKING AND DEWATERING PROJECTS

Permanent shoreline improvements in the Kentucky Reservoir include diking and dewatering of 5,000 acres (3-foot zone) on which current costs are approximately $15 per acre, which compares to a conservative figure of $25 per acre under conventional but less effective antilarval methods.

There are eight diking and dewatering projects in the Kentucky Reservoir, ranging in pumping capacity from 16,000 gpm to 250,000 gpm. Four of the larger projects, namely, Duck River, Big Sandy, West Sandy, and Camden are located in the middle portion of the impoundment. The remaining smaller projects are located in the upper portion of the reservoir. Pertinent information relative to each of the eight projects is listed in Table I.

Agricultural Benefits

These projects embrace relatively large areas where the control of mosquitoes by the use of conventional methods would have been very difficult and expensive. Although they were constructed primarily for malaria control, they have had many collateral uses since completion. The uses include production of agricultural crops, timber, fish, and wildlife. In addition, recreational activities in and around these projects have been intensified as a result of their construction. The value to the

licensees of agricultural crops produced in the eight dewatered areas in 1948 is estimated to be $72,000. The value of crops left in the fields for food for wildlife is estimated to be $41,500. Acreages are somewhat misleading, since some of the land was

TABLE I

Pertinent information relative to diking and dewatering projects in Kentucky reservoir

PROJECT	SIZE OF PROJECT IN ACRE	MILES OF SHORELINE	LENGTH OF LEVEE IN FEET	LENGTH OF DRAINAGE DITCHES REQUIRED IN FEET	TYPE OF PUMPING PLANT	CAPACITY OF PUMPING PLANT (GPM)
Duck River	4,688	34	46,500	116,219	Gasoline	171,000
Camden	2,807	19	34,200	69,918	Electric	145,000
Big Sandy	1,738	12	21,000	45,340	Gasoline	30,000
West Sandy	3,730	38	3,600	168,668	Electric	250,000
Perryville	308	9	3,200	18,122	Gasoline	24,000
East Perryville	223	15	2,600	32,246	Gasoline	16,000
Busseltown	204	13	600	12,875	Gasoline	18,000
Gumdale	152	7	2,600	34,067	Gasoline	18,000
Total	13,850	147	114,300	497,455		672,000

TABLE II

Agricultural benefits from dewatered areas Kentucky Reservoir 1948

PROJECT	ACRES PLANTED WHICH LICENSEES HARVESTED	GROSS VALUE OF CROPS PRODUCED AND HARVESTED	NET ECONOMIC VALUE TO LICENSEES	ACRES PLANTED AND CROPS LEFT FOR FOOD FOR WILDLIFE	VALUE OF CROPS FOR WILDLIFE	NET RENT PAID TVA	COMMISSIONS PAID LAND-USE ASSOCIATIONS
Duck River	2,274	$72,513	$50,985	1,836	$24,575	$2,307	$977
Big Sandy	128	3,175	2,193	164	4,920	147	43
West Sandy	130	2,065	1,414	259	7,680	55	55
Camden	807	16,596	9,457	100	3,000	2,547	449
4 Smaller Projects	532	12,862	7,772	55	1,320	1,485	269
Total	3,871	$107,211	$71,821	2,414	$41,495	$6,541	$1,793

Agricultural values were taken from reports submitted by the Land-Use Associations, and both yields and crop values are considered conservative.

Estimates based on (corn $1.00 per bushel; soy beans $10.00 per acre; milomaize $25.00 per acre) current crop values less cost of depreciation on machinery and tools, the cost of corn, milomaize and buck wheat seed and other expenses which take money from the community.

double cropped, that is, corn and soy beans were planted together. However, out of a total of 13,850 acres inside the dike, there were 3,871 acres of conventional farm crops planted and harvested, while 2,414 acres were planted to food for wildlife. Details by projects of acreages and basis of estimated values are shown in Table II.

Many of the collateral benefits from dewatered projects are made possible by close

adherence to operating schedules for each project prepared annually prior to the beginning of the mosquito breeding season, usually in January. In developing these yearly operating schedules for these projects, full consideration is given to other program interests and needs when they do not interfere with those for malaria control. The operating schedule on the Duck River project for the calendar year 1948 is an illustration of how agricultural, forestry, and wildlife interests were incorporated into the operation of Duck River. The area was dewatered to elevation 352 by May 1, giving ample time for the planting of agricultural crops on most of the available land. Furthermore, timbered areas were dewatered during the growing season, which promoted optimum growth.

Forestry Benefits

Due to the fact that the timber in these areas is predominantly young or medium in age, the income from timber harvesting will be low for a number of years. However,

TABLE III

Estimated average annual timber values from forested areas within diking and dewatering projects Kentucky Reservoir

PERIOD	ACRES IN TIMBER	ANNUAL CUT MBF	VALUE TO THE TIMBER OPERATORS AND EMPLOYEES	STUMPAGE VALUES TO TVA
1950–1995	6,000	750*	$30,000*	$9,000*
1995–	6,600	1,860	74,000	22,000

* These figures represent the estimated average annual amount for this 45-year period. Actual values will be lower than these at the beginning of the period and higher toward the end.

Note: Stumpage values are based on an average of $12 per thousand board feet. Values to timber operators and employees are based on an average of $40 per thousand board feet. This includes costs such as felling, skidding, hauling, milling, and mill operators' profits. It is assumed that payment received for these items would be left in the reservoir area.

under good forest management, the annual income can be built up to a very significant sum. Table III indicates the estimated average annual yield in board feet and the corresponding monetary values that can be expected from forested areas within the diking and dewatering projects. Without diking and dewatering, these productive timbered areas would have been cleared of their natural growth and flooded with the impoundage of Kentucky Reservoir.

Recreational and Other Benefits

It is difficult to measure the value of the malaria control projects with respect to the recreation program; however, it is evident that the growth in person-day visits and in recreation investments is influenced greatly by the effective treatment of the mosquito problem.

Representatives of conservation interests indicate that hundreds of thousands of ducks have used Kentucky Reservoir and the dewatering projects in the past few years that would never have visited the region had it not been for the dewatering areas. At the same time, thousands of hunting days have been spent in the field by

the sportsmen, and the success of these hunts has been excellent. By far the heaviest concentration of waterfowl and duck hunters in the region is to be found in the dewatering projects.

Data for Table IV were furnished by a representative of the Tennessee Department of Conservation, giving pertinent information concerning areas where shooting of ducks is permitted.

DEEPENING AND FILLING PROJECTS

During the preparation of Kentucky Reservoir for impoundage, a decision was reached that in certain areas malaria control could best be accomplished by the use

TABLE IV

Duck hunting data
Kentucky Reservoir
Season of 1948–1949

AREA HUNTED	NO. MAN-DAYS HUNTED	TOTAL DUCKS KILLED	TOTAL DUCKS CRIPPLED	TOTAL GEESE KILLED
West Sandy	1,800	3,690	882	0
Big Sandy	400	332	104	4
Camden	360	385	158	0
Perryville, Gumdale, East Perryville	300	300	100	0
Other Areas	400	200	75	3
Totals for Ky. Reservoir	3,260	4,907	1,319	7

The above figures are based on actual tallies and contacts with 103 hunters and a fairly close count of daily hunters in the West Sandy, Big Sandy, and Camden areas. The representative also made the following statement: "It is the opinion of the undersigned that a minimum of $5.00 per duck killed or crippled was expended by hunters in the Kentucky Reservoir area; thus, an outlay of funds amounting to $31,130 might be considered as having been left in this area, considering the normal expenditures for gas, shells, licenses, hunting clothes, motor boats, decoys, calls, liquor, hotels, etc. This is probably a very small figure."

of the deepening and filling process. At that time, there were eight areas[2] involving 1,662 acres worked by this process. Of this acreage, 881 acres were in the fill section, which was constructed to elevation 359.3 feet, or three-tenths of a foot above the normal summertime operating level of the lake. Subsequent to impoundage, a number of smaller areas in the middle portion of the reservoir have been treated by this process, and the area in the newer fill sections amounted to 90 acres.

Inasmuch as the land sale policy on Kentucky Reservoir provides for the indefinite retention by TVA of all land now owned below elevation 375 and since all of the filled sections are below that elevation, it appears that some long-term management of these areas should be considered. Values or benefits concerning these projects are discussed below:

[2] Namely, Blood River, Jonathan Creek, Eagle Creek, Swamp Creek, Beech River, Hardin's Landing, Clifton, and Cypress Creek.

Agricultural Benefits

Although fairly large areas of land became available as the result of the deepening and filling program in Kentucky Reservoir, relatively little use has been made of these areas from an agricultural standpoint. This is due to the fact that TVA has not limed, fertilized, and seeded these areas as would be required to condition them for pasture or crop use. However, in the upper portion of the reservoir, some of this work was done on land where TVA had acquired easement rights only. The land owners realized the potentiality of the area built out of the reservoir, and did the necessary improvements to make the land into a valuable pasture. It is reported that practically all of the land in the fill areas may be made quite productive if lime, fertilizer, and seed are added after the completion of construction of the fill.

Forestry Benefits

None of the filled areas has been reforested to date. These bottomland sites can be made productive through reforestation. Tree growth is rapid on lowland types such as is common to the filled areas. Over a long period of time, it is estimated that an average annual income of about $5.00 per acre could be realized if the areas were planted with forest tree seedling stock. The cost of planting would be approximately $12.00 per acre.

Recreational and other Benefits

There are a number of intangible benefits to recreation resulting from the deepening and filling program. These may be listed as the elimination of unattractive mud flats, improved mosquito control, provision of better boat access at normal lake elevation, and the elimination of the need for supplementary mosquito control measures in areas receiving extensive recreational use.

The deepening and filling program as carried on by TVA at the heads of the shallow bays of the reservoir has no current value in connection with the wildlife program. On the other hand, the fill does create an area of real possibilities for the development of goose pastures. At this stage of the development of Kentucky Reservoir, these areas remain undeveloped for and unused by geese. This developmental phase of the waterfowl program on Kentucky Reservoir has only started, and no doubt as the program expands, these areas will take on a greater value.

MARGINAL DRAINAGE PROJECTS

During preparation of the reservoir, construction of marginal drainage ditches was necessary in order to eliminate certain isolated pools which would form as the reservoir receded. Some of these ditches extended into the flood surcharge zone, and although constructed for malaria control purposes, they are also of value for agricultural purposes. There are a number of fields now used for the production of crops that had not been used for this purpose prior to the construction of the drainage system, but a realistic estimate of the value of this outgrowth of the malaria control work cannot be made from available records.

In addition, these marginal drainage projects have had some value from the

recreational standpoint. This is due to the fact that the ditches improved access to areas considered as good fishing areas, and they made available several isolated pools which are suitable for boat harbors. Two of these are being developed.

PERMANENT GRAZING AREAS

During the early stages of the impoundment of the reservoir, the establishment of malaria control grazing areas was considered, the idea being that grazing of the reservoir margins would maintain conditions unfavorable to mosquito breeding by keeping the shoreline relatively free of vegetative growths. Initially, eleven of fifty-one areas studied were fenced as pastures. However, they have not all been grazed to the extent anticipated, and the extension of this method of control is not anticipated. Further, the development of upland pastures by the farmers on their land has diminished the demand for rental pastures remotely situated. In view of this, areas formerly studied and proposed as grazing areas have been included in those proposed for treatment by the deepening and filling process.

SMALL BOAT CHANNELS AND OPERATING BASES

Early methods of larviciding used by TVA included application of larvicidal oil from boats and of certain dusts from both boats and airplanes. To facilitate these boat operations, channels were marked to aid navigation into shallow flat areas. However, with the advent of the use of DDT as a larvicide, the application of dusts and oil was discontinued in favor of the more effective and more economical application of DDT by airplane. With this change, the navigation channels previously marked as aids to the malaria control program were no longer needed in connection with this program; however, these channels have proved very valuable to fishermen and other small boat users of the lake. They serve as boat access to certain land developments along the lake shore, and they provide access to protected harbor areas that are used quite extensively by small craft when navigation on the main or open lake is hazardous.

Before the use of DDT was developed, when larvicides were applied by boat, a small fleet which required eight bases was operated. In the selection of sites for these bases, consideration was given to the possibilities for joint use of access channels with adjacent recreation boat dock sites and to the further possibility that when they were no longer needed for malaria control use, they might be used as recreation boat docks. They were placed at strategic points along the shoreline with respect to mosquito breeding areas in order to minimize travel time and the attendant expenses involved in getting from one problem area to another. The care in selection of these sites was justified because with the program change from oiling and dusting from boats to the application of DDT by airplane, all but one of these bases were sold or leased to commercial boat dock operators. For the most part, the docks have been operated commercially for less than a year, and an estimate of their gross revenue would be only a guess. On the other hand, business is reported as being good and is expected to increase with further development. An example of these converted operations is Jonathan Creek Malaria Control Boat Base at Lucas' Harbor.

LAND USE RESTRICTION

Early studies of the malaria control problem areas of the reservoir indicated that one section of the reservoir just above the mouth of Duck River would present difficult, if not impossible, problems for the control of malaria by conventional methods, and costs for permanent shoreline improvement would have been prohibitive. This section was characterized by expansive shallow flat areas and a sparsely settled shoreline. The average population per mile of shoreline was six, compared to about one-hundred per mile in some portions of the impoundment. Land contiguous to the shoreline was mostly "cut over" timber land with a second growth having relatively low values, but there was also a small amount of open land in cultivation and pastures. It is estimated that at least 90 per cent of this open land not flooded by the reservoir is still used in this manner. The easement carries only the right to prohibit night-time occupancy of the area during malaria mosquito breeding period. Therefore, by living relatively close to the area, farmers are able to carry on their usual operations.

SUMMARY

During the preimpoundage studies of Kentucky Reservoir, it was evident that, due to its size, flat topography, and certain limitations on water level management, the conventional malaria control methods would not suffice. In recognition of this, limited areas were specially treated with diking and dewatering, deepening and filling, and land-use restriction. Although these projects were constructed primarily for malaria control, they have had numerous collateral uses, such as crop production, wildlife development, and the enhancement of recreational values. Furthermore, the full development of these areas will increase their worth. For instance, the fill areas of the deepening and filling projects may be made quite productive by reforestation, and some of the diking and dewatering projects can be made more desirable from a wildlife standpoint.

Currently, the diking and dewatering projects are producing the most benefits because their collateral uses are more diversified. A dollar value can be placed on some of these benefits, but there are other intangibles on which a value would be difficult to estimate. Crops harvested by the farmers on eight such projects had an estimated value of $72,000, that left for wildlife was estimated at $41,000, and TVA received cash rentals amounting to $6,500 in 1948. The forestry benefits, beginning at maturity of the present timber growth, approximately twenty years hence, would have an estimated annual value of $78,500 to the timber operators and employees and a stumpage value to TVA of $23,500 annually.

Other malaria control features and facilities, such as marginal drainage projects, malaria control grazing projects, and malaria control bases, have contributed substantially to the economy of the region. However, some of the benefits are intangible; therefore, no estimate of their worth is presented. On the other hand, there are real values that can be realized from the fill areas of the deepening and filling projects if put to use.

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SUMARIO

Los estudios que precedieron al almacenamiento de las aguas en el Embalse de Kentucky indicaron con toda evidencia que debido a su tamaño, topografía plana y ciertas limitaciones para el manipuleo del nivel de las aguas los métodos convencionales de control de malaria no iban aser suficientes. En reconocimiento de esta circunstancia algunas áreas fueron especialmente tratadas mediante diques perimetrales y bombeo, canalización, rellenos y restricciones en el uso de la tierra. Aunque estos proyectos fueron principalmente ejecutados para control de malaria, ellos han tenido numerosos usos colaterales tales como producción de cosechas, desarrollo de vida silvestre y el acrecentamiento de facilidades de recreación. Además el desarrollo completo de estas áreas aumentará su valor. Por ejemplo, las áreas recuperadas de los proyectos de canalización y relleno pueden hacerse productivas mediante forestación, mientras que algunos de los proyectos de diques y bombeo han cuasado un mejoramiento de la vida silvestre.

En general los proyectos de diques y bombeo producen los mayores beneficios a causa de que sus usos colaterales son muy diversos. Un valor monetario podría considerarse entre estos beneficios pero hay otros valores intangibles difíciles de estimar. Cosechas obtenidas por los hacendados en ocho de estos proyectos han sido estimados en $ 72.000, el beneficio para la vida silvestre fué estimado en $ 41. 000 y la Autoridad del Valle de Tennesse recibió rentas que montaron a $ 6.500 en 1948. Los beneficios forestales que comenzarán con la madurez de las maderas, después de unos 20 años tendrán un valor anual de $ 78.500 a los operadores y empleados en su explotación y un ingreso por derechos de explotación de $ 23.500 anuales para la Autoridad del Valle Tennesse.

Otros elementos y facilidades debidos al control de malaria, tales como proyectos de drenajes en las márgenes, limpieza de vegetación y el establecimiento mismo de las bases han contribuido subtancialmente al mejoramiento de la economía de la región. Sin embargo, algunos de estos beneficios son intangibles y por consiguiente no se presenta estimación de su valor. Por otra parte, hay valores reales que pueden hacerse evidentes mediante el aprovechamiento de áreas rellenas y drenadas.

FIELD STUDIES ON THE BIONOMICS OF *ANOPHELES ALBIMANUS*

PARTS II AND III: DIURNAL RESTING PLACES—PROGRESS REPORT[1]

JOHN W. H. REHN[2], J. MALDONADO CAPRILES[3] AND JOHN M. HENDERSON[4]

PART II—J. W. H. REHN AND J. M. HENDERSON

This is the second (Rehn *et al* 1950) of a series of reports concerned with field observations on the anophelines of Puerto Rico. The general studies were initiated in September, 1948 by the senior author, in resident charge in Puerto Rico, and by the junior author, who is non-resident administrative director of the project. The other principal participant in the investigations here reported was J. Maldonado Capriles, who separately undertook investigations on the west end of the island, as described in Part III.

The overall studies include investigation of a number of lacunae in the bionomics of anophelines in Puerto Rico, particularly *Anopheles albimanus*, which have significance in the control of malaria and the control or eradication of anophelines in this and other areas.

Probably the most important single lacuna in the knowledge of the bionomics of *A. albimanus* and of the two other indigenous anophelines (*Anopheles grabhami* and *Anopheles vestilipennis*), pertains to their customary diurnal resting places in Puerto Rico. The demonstrated vector of malaria, *A. albimanus*, with rare exceptions, departs from the habitations of man and animal during the night or at the latest, by dawn, and thereafter the location and habits of the adults of both sexes during the daytime hours are not known. Since knowledge of the diurnal resting places of this species, especially the female, has important potential significance in species control and eradication, this subject was given overriding priority in the program of investigation during the season of abundance in 1949.

All mosquitoes may be found resting for brief periods after emergence at the breeding place. Since our interest lies in the behavior of the adult of this species over its general life span, and particularly of the engorged and/or gravid female, attention was focused on the vicinities of feeding places.

Other investigators have attempted to determine the favored daytime resting places of *A. albimanus* in Peurto Rico and elsewhere. Scattered, unpublished information on such observations in Puerto Rico are known to us. It is reported that Earle (W. C.) and his co-workers at times found them, apparently unengorged, in natural outdoor resting places, but never in numbers sufficient to indicate the large popula-

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tion known to be present at the time, as indicated by stable trap collections. On the north coast of the island, adults were found fairly consistently by Pratt (H. D.) in a dairy at Villa Palmeras and at one time by Pérez Torres (M) in buildings at old Loiza, but they were unable to find adults in similar situations elsewhere, even when populations at other places were substantially greater. In spring of 1949, occasional specimens were found by another observer (Mr. Griffith, U. S. Marine Corps) in houses and other buildings on the nearby island of Vieques. Le Prince and Orenstein (1916)[5] reported the finding of large numbers of *albimanus* on the leeward side of trees in Panama near extensive breeding places.

From an examination of the literature and through correspondence and conversations with investigators acquainted with this species in Central America, it would seem more inclined to rest in buildings during the daytime in those areas than has ever been observed in Puerto Rico. Matheson and Vargas have found the species moderately common in houses in eastern Mexico in morning examinations (unpublished) and the former mentions this habit in Medical Entomology (Comstock Press, 1950). Komp, (W. H. W.) and Rozeboom (L. E.) have mentioned that they have collected this species in habitations. Trapido (H.) in Panama has at times found *A. albimanus* in buildings in the daytime (personal discussions).

OBSERVATIONS

The search for diurnal resting places on the present study was carried on in two phases. The first was between January and May, 1949, when populations generally were sparse, and consisted of making sporadic field observations in conjunction with visits to dry and wet breeding places for reconnaissance and for the collection of soil samples in connection with the possible aestivation of immature stages. These visits were made along the relatively arid south coast of the island, and were substantially unproductive except for one area in the vicinity of Central San Francisco where *Anopheles* were occasionally abundant.

Within one-quarter mile of a breeding place under study in this area is a rough limestone cliff, with faults, crevices and cracks. The breeding place itself was in the midst of a heavily grazed pasture, in which cattle remained overnight. On the vertical surface of the cliff, *albimanus* adults were found resting in small to moderate numbers; up to 30 individuals were observed in one-half hour, with lesser numbers on several other occasions. They were in relatively exposed positions but not in direct sunlight. The entire cliff was protected from direct wind. Individuals were found scattered from the base to elevations of at least 20 feet, males being more abundant than females. The relative humidity at resting points was in the upper 80's. Individuals were never found in the crevices or other protected, dark and more humid situations. A survey of the under sides of small limestone rocks, each with approximately 1 sq. ft. of sheltered surface and located near the water's edge, along a fence and scattered on the gound, revealed *albimanus* resting in moderate numbers. On one examination up to 3 per rock were found; on another an average of nearly 1 per rock.

[5] Le Prince, J. A. A. and Orenstein, A. J., 1916: Mosquito Control in Panama. New York. G. P. Putnam's Sons. 335 pp.

They were always on the lower surfaces, thus being protected from direct sunlight. In all cases where the relative humidity was recorded, it was in the upper 80's.

An examination of several caves of various sizes and with a variety of exposures and types of openings in this same area was not productive. Only a single recently engorged *albimanus* was found; this was about five feet above the floor level on a spider web in one of the larger caves. In the same cave, one *A. grabhami* and one *A. vestitipennis*, both engorged, were found.

Searches of the undersides of leaves of terrestrial and mangrove bushes and trees within $\frac{1}{2}$ mile of the breeding area were made with negative results although adults of various *Aedes* species were abundant. Scattered adults were found on exposed portions of roots, in wide forks of trees and in other relatively exposed situations. Individuals were not found in direct sunlight nor exposed to wind currents; neither were they in the most sheltered and darkest situations.

The island of Puerto Rico is approximately 100 miles long and 30 miles wide. Because of a chain of high hills and mountains which occupies the interior of the island, anophelines are mainly found along the coastal periphery. The second phase of the study was undertaken from June to December 1949, and was confined to the east, north and west coasts of the island, since these areas were more accessible than the south coast to the investigators, who were located respectively at San Juan, on the north central coast, and at Mayaguez, on the west coast. In this phase, it was decided to undertake extensive investigations of possible and potential natural resting places in areas on the north coast having a known high population. Major attention was given to the areas surrounding relatively well-isolated blood meal sources near heavy breeding places. Initially, three sites were selected; one was soon eliminated, while a second had such fluctuations in populations that studies there were discontinued. The remaining station, which was physically and ecologically most suitable, was the area most intensively examined. This was a mixed pasture land-cane field area in the vicinity of a stable at Central San Vicente, in Vega Baja Municipality. A nearby secondary area was also included later in the season. Extensive reconnaissance surveys were made for other suitable areas on the north and east coast, and on Vieques Island, but none was selected, due to lack of concentrated and relatively isolated sources of blood meals, inadequate numbers of adult *albimanus*, or both. Investigations were made on the west coast in the vicinity of Mayaquez by Maldonado, as reported in Part III. The areas involved in his studies were ecologically different from those studied on the north coast.

The north coastal areas studied in detail were either sketched, or large scale existing maps of them were obtained, and localized ecological conditions were entered on them. In other areas the sites were roughly sketched and the existing conditions were described in notes.

The problem was approached by examining all possible or potential resting places in the vicinity of the blood sources. It is believed, from the knowledge available for other species of *Anopheles*, that there may be an initial diurnal resting period not far removed from the feeding place. The duration of this resting period is unknown, but is thought to range from several hours to a day or more.

Keg-style artificial resting places of a type successfully used with *Anopheles*

quadrimaculatus had been previously tried on a limited scale in a number of situations, with negative results. Kegs were placed in vegetable gardens and in pasture, shaded and unshaded, near and remote from stables, near the edge or in the center of cane fields and along ditches and stream banks. The openings were oriented in various directions and the kegs were placed on the ground or partially buried, and were at times either partially packed or covered with vegetation or moist soil.

Sweeping and beating of vegetation, were done in a number of ecologic situations, but *Anopheles* were found by this method only in the Mayaguez area (see report by Maldonado). The following types of situations were examined in the locations previously discussed: 1) short grass, 2) tall pasture, 3) fallow fields (mixed grass, weeds, and shrubs), 4) low sugar cane, 5) low shrubs, 6)mixed vegetation on stream and ditch banks, 7) bamboo thickets. Both shaded and unshaded areas in types 1, 2, 3, 5, and 6 were examined. Numerous and extensive examinations from May through September were made at different times a day. Beating and sweeping were also interspersed with the types of observations noted below.

Searches were made extensively, but with negative results. These were carried on in all of the above mentioned situations, and also in the following types of locations: 1) tree and bush trunks and limbs, and foliage that could not be beaten, 2) ground litter and soil surfaces in pastures and in sugar cane fields, 3) plants and ground surfaces in vegetable gardens, 4) banana and plaintain groves, 5) in and particularly under, buildings, including ground surfaces, 6) culverts, drains and bridges, 7) stream, pool and ditch banks, 8) caves, 9) under stones and rocks, 10) on and under various types of equipment.

In some of these situations thorough coverage was possible, but in areas that had dense vegetation it was impossible to be sure that anopheles were not disturbed or overlooked. However, in these situations the finding of *Aedes*, *Culex* and other genera of Culicinae as well as Culicoides and numerous other small Diptera would seem to indicate that if these were preferred resting placed for *albimanus*, some individuals would have been found.

As by either sleeping and beating or by visual searches practically all ecologic situations in the vicinity of the blood sources had been examined with negative results, other methods of approach were indicated. Since pasture land seemed promising as a resting place, a limited number of tests was first attempted to determine if recently engorged individuals survived in this habitat.

The following methods were utilized: 1) leaving the preceding night's collection of specimens in stable traps installed in pasture until late afternoon (predatory ants were usually present, and at times both spiders and lizards would enter the traps). 2) releasing *albimanus* adults collected elsewhere into newly stationed stable traps, either with or without the entrance slots closed (the only predators particularly excluded by this procedure were lizards). 3) releasing individuals under an erected mosquito bednet, with only the lizards removed from the area. In all cases where lizards were excluded, it is believed that the artificial structures gave a better opportunity for the capture of mosquitoes by this predator than would exist in the grass under natural conditions. In these tests ants were usually common and spiders and various predaceous Hemiptera usually were present. By the first and second methods

results were encouraging, but as there was some protection from climatic conditions, the third method was tried before it was believed that survival was definitely possible and probable. With releases in bednets, recaptures of living mosquitoes after a twenty-four hour period varied from 30 per cent to more than 70 per cent, usually between 50 and 70 per cent. The lower figures were obtained when there had been relatively high winds and considerable rain during the test period.

Following these findings it was decided to make additional attempts to find *albimanus* in pastures, fallow fields and sugar cane areas. A method of placing mosquito bednets over an area without disturbing the vegetation, and then attempting to disturb and drive up flying insects in the enclosed area was devised. Four corner poles ($\frac{3}{8}$ inch steel rods), were used to handle the net. One person at each end lifted the net and lowered it over the area to be examined without disturbing the vegetation in the test area. At times the tops of high plants were touched but this was avoided whenever possible. Short pieces of wood or metal were fastened to the bottom edges of the net as weights. At first large bednets were used and the poles were fastened to the corner ties; it was soon found more advantageous to run light rope through the net and tie to the poles; thus the net was suspended without strain. To increase the size of the bednet enclosure, sections were inserted in the middle of the net. This was found to be impractical because of difficulty in placing the enlarged net over the test area without disturbing the vegetation.

The enclosed vegetation was sprayed with a solution of cyclohexanone and water, which was an effective excitant or irritant, as many types of insects emerged after spraying from the vegetation and rested on the netting. Agitation of the vegetation after spraying was found to increase the numbers of insects found. "Shur-shot" sprayers (Model A, gross capacity $1\frac{1}{2}$ qts.), were found to be satisfactory for the application of cyclohexanone. A mixture consisting of 5 cc. of cyclohexanone and approximately one quart of water was placed in the sprayer and then air-pressure was built-up. Dosages were not accurately measured due to differences in density of the pasture grass, but sufficient material was used to moisten the vegetation and soil surfaces. It was found by experimentation that the dosage used did not knock down *albimanus* or apparently otherwise injure them, as survival among those exposed was comparable to that obtained in controls. The quantity of cyclohexanone in the mixture was at times increased to 10 cc., without apparent change in the reactions of insects exposed. Initially, pressure was supplied to the sprayers by adding compressed air at a nearby automobile filling station. The sprayer was loaded to a pressure of 60 to 75 psi, depending on its condition, as at higher pressures the gaskets soon developed leaks and needed frequent replacement. The spray charge was applied as long as a good mist spray was delivered. This usually would allow the coverage of six to eight tests areas before the pressure failed. Under these conditions approximately one-half the liquid volume would be expelled. Subsequently, in December, the sprayers were charged from a cylinder of compressed carbon dioxide, but this was not entirely satisfactory, as fewer insects of all types were disturbed when this method was used. (We have learned subsequently that CO_2 is an insect anesthetic). Aerosol-type bombs were considered, but were not used. The use of freon as a propellant was also considered but not attempted.

By the method outlined above an area of 18 to 20 square feet of surface was examined during each bednet "run". Although considerable quantities of Culicine mosquitoes and other insects were found, only a few *Anopheles* were encountered.

DESCRIPTION OF SAN VICENTE STUDY AREA AND TRAP COLLECTIONS

The San Vicente Study Area had as its central feature a large, isolated stable, housing about 25 horses and usually two to four draft steers. Adjacent to the stable

TABLE I

Summary of All Collections by Bednet

MONTH	NO. BEDNET RUNS	TOTAL ANOPHELINES	A. ALBIMANUS (FEMALE)
August	24	3[2]	2
September	46	6	4
October	43	0	0
November	36	2	1
December	49	0	0
Total	198[1]	11	7

[1] Includes 36 "runs" in cane fields at San Vicente and 20 in pasture near Esperanza, Vieques Island. All 56 were negative. Cane was 2 to 4 ft. high.

[2] Includes 1 *albimanus* (male).

TABLE II

Collections by Bednet in Pasture Area—San Vicente

MONTH	NO. BEDNET RUNS[1]	TOTAL CULICINES[2]	TOTAL ANOPHELINES	A. ALBIMANUS (FEMALE)
August	24	40	3	2
September	20	25+	6	4
October	33	35+	0	0
November	26	74	2	1
December	39	21+	0	0
Total	142	195+	11	7

[1] All "runs" through November were with compressed air. December "runs" were with CO_2. Total ground area examined by months: 456, 380, 627, 494 and 885 sq. ft.

[2] Predominant genus varied seasonally. Total collection included 1 Mansonia, remainder were Psorophora, Uranotaenia and Culex, in approximately equal numbers.

on three sides were three pastures, with a combined area of 13 acres. The horses, and at times oxen and cows, generally were kept in the pastures at night. All bednet "runs" reported in Table II were made in these pastures, but *albimanus* were collected only from one pasture that was most commonly used at night. The distance of the bednets to the stable varied from 50 feet to over 2500 feet, but in many cases they were at or within 25 feet of places where animals had bedded in the pastures during the preceding night.

The dominant vegetation in the pastures was para grass, (*Panicum*) which occurs

extensively in the interior valleys and coastal plains of Puerto Rico. This grass varied in height from 6″ to 24″, providing only moderate cover where trampled on or intensively grazed, or in wet spots, and dense cover wherever it occurred in thick clumps. The few anophelines found tended to select the thicker and higher clumps, and usually the moister situations, while the culicines (except *Uranotaenia* were more generally distributed. With a very few exceptions, collections were made in the morning hours before large numbers of mosquitoes could be destroyed by predators.

In cane field areas, land reserved for pasture is generally too poorly drained for the cultivation of sugar cane. The study pastures were wet and hummocky, with many small groundpools concealed in the grass during wet weather. During the period of the study, the pastures were intermittently dry and wet. In the second half of November and in December, rains were so frequent as to keep the foliage wet in the daytime. This matted it and tended to seriously impair the effectiveness of the spraying, but at no time was the extent of surface water over the general pasture areas sufficient to eliminate thick grassy harborage for adult mosquitoes.

Relative humidity readings were made with a Friez Hand Aspirated Psychrometer (Model H/2) on all occasions when *albimanus* females were collected. Since the elevation above ground level at which these individuals were resting was not known, readings were made at ground level and in the grass at distances varying from 2 inches to 24 inches above ground level, depending on the height of the grass. The relative humidity varied from 80 per cent to 92 per cent; the mean of 7 readings were 86 per cent. The mean of four readings made in shade at the top of the grass was 83 per cent, while that of three readings made at or near the surface of the soil was 89 per cent.

As indexes of *albimanus* populations, an electric light trap and a stable trap were operated throughout the study period. The electric light trap (New Jersey type), was installed immediatly outside of the stable, while the stable trap (modified Magoon type) was installed in the pasture. Collections of *albimanus* by light trap (40 overnight collections), varied from 3 to 535. The mean numbers of *albimanus* collected per "run" for the 5 months August–December, 1949 were 239, 75, 136, 55 and 52 respectively. Ten stable trap collections were made within the same period. Excluding November, when the trap was not operated, comparable collections were 143, 112, 38,—, and 31; varying from 12 to 266 per "run". The combined average for the total period and for both traps was 98 *albimanus* per "run". On basis of many years experience by personnel of the Insular Health Department and the U. S. Public Health Service with these traps, operated in conjunction with malaria control projects, these collection results are considered as representing an abundant population. (It has been found necessary to reduce to an average of one mosquito per trap per night to interrupt malaria transmission in Puerto Rico).

PART III—J. Maldonado Capriles

A. FIELD SEARCHES

The following places were inspected visually for adult anophelines: 1. Short-grass associations under cocoanut palms; 2. Water hyacinth (*Piaropus*) on river margins;

3. Cat-tail (*Typha*) swamp; 4. Para grass (*Panicum*) marsh; 5. Short sugar cane fields (2–5) feet high); 6. White mangrove (*Laguncularia*) swamp; 7. Mangrove fern (*Acrostichum*) swamp; 8. Banana trees; 9. Coconut halms; 10. Grapefruit trees; 11. Under bridges; 12. Barns and stables.

More attention was given to items 5,6,7,9 and 11, especially item 5 (sugar cane fields). Only a few anophelines (all *Anopheles grabhami*), were seen resting on cocoanut palms. This is detailed in the following table:

Examination of Cocoanut Palms

	No. of Palms examined	*No. of A. Grabhami*
Santa Ana, June 21	8	1
July 13	6	2
July 15	11	3
Asad Dairy, July 21	12	0
	—	–
Total	37	6

Careful search was made of the trunk and leaves of each palm. The observations of July 15th were made up to 14 feet above the ground and on the other dates up to 8 feet. During the above period the prevailing *albimanus* population was slightly higher than the *grabhami* population, but only female *grabhami* were observed resting on the palms. These few specimens were found in places both protected and unprotected from breeze and sun.

Observations in cane fields were carried on by assiduously searching the ground, grasses and the entire sugar cane plants. At Santa Ana search was made of fields with ditches in which there was moderate breeding of *albimanus* (5–10 larvae of all sizes per dip). At the Industrial School for Boys, sugar cane located 100–200 feet from a light trap that had collected 309 *albimanus*, 1,451 *grabhami* and 14 *vestitipennis* a few days before, was searched for more than 3 hours. By lightly shaking the plants resting insects were made to take wing. Although some culicines were seen, no anophelines were noticed.

Search in the other places mentioned above, except for a few anophelines seen in the Asad Dairy, yielded negative results.

B. SWEEPING.

Two ecologic situations were thoroughly swept for anopheline adults—sugar cane fields and short-grass associations under cocoanut palm shade. About 24 hours were devoted to sweeping cane plants and dead leaves and grasses, especially "cohitre" (*Commelina*), found under the cane plants. Humid places, close to a source of blood meal, were selected for this work. At Colonia Lange, search was made from 25 to 75 feet around a stable, where the night before over 100 *albimanus* were observed resting and feeding, and also along a road, covered with medium height grasses, leading from the stable to a mangrove swamp about 350 feet away. At Santa Ana, 5 hours were spent in searching under almost identical conditions except that heavier breeding was occurring in the ditches. No anophelines were seen or captured in the cane fields at either place. At Colonia Lange, two engorged female *grabhami* were seen resting on a fallen log, and one female *albimanus* was swept from a hanging vine.

Sweeping low vegetation under cocoanut palm shade at three different places produced some *albimanus* and *grabhami* of both sexes, as shown in Table III.

At Vaqueria Asad and Santa Ana collections were made from grasses under cocoanut palms and citrus trees, and at the Reform School from a bed of short sunflowers. The first two places were almost identical with regard to shade, apparent soil humidity, and dominant species of short grasses. Both places also had a plentiful source of

TABLE III

Anophelines collected by Sweeping

		ALBIMANUS		GRABHAMI		RELATIVE HUMIDITY[1]
		Male	Female	Male	Female	
Vaqueria Asad	July 21	10	5	5	16	91%
	25	5	3	4	2	85
	30	0	2	0	0	—
Santa Ana	July 22	14	13	2	2	80
	25	6	3	3	2	—
Industrial School for Boys	July 21	0	0	0	1	—
Total.....		35	26	14	23	

[1] Humidity readings are not accurate, since they were taken by sling psychrometer above the swept vegetation.

TABLE IV

Anophelines under house, Santa Ana

DATES	ALBIMANUS		GRABHAMI		NOTES
	Male	Female	Male	Female	
July 6	0	0	1	14	at 2 PM
8	0	0	0	8	at 11 AM
11	0	0	0	5	R.H. 51%
13	0	0	1	3	R.H. 63%
15	0	0	0	5	at 9–10 AM
22	0	3	1	11	"
Aug. 2	0	0	0	8	"
Total.....	0	3	3	54	

animal blood meals close by. An electric light trap at Vaqueria Asad was collecting between 250 and 290 *albimanus* per night. It is noteworthy that at Santa Ana search was made by sweeping close to the source of blood (from grass) and close to one of the breeding places (short cane), but anophelines were found only in the first location. The cocoanut grove at Central San Francisco, Guayanilla, with very similar conditions, except from a lower humidity, produced no anophelines. Recovery of adults from the sweeping net was difficult and the specimens were difficult to identify as they were damaged. All these places, at Mayaguez and Guayanilla, were checked several times from September to December, but no specimens were collected.

During July and August anopheline adults, principally *grabhami* females, were frequently seen resting during the daytime under a house at Santa Ana, as shown in Table IV.

Overnight electric light trap collections at this place were low. The highest collection was 30 *albimanus*, and *grabhami* were consistently fewer. About two-thirds of the specimens seen were engorged. Anophelines were swept from grass around this house.

At Colonias Lange and Altagracia no anophelines were found under houses during the day at any time, although moderately high numbers of anophelines were seen resting at the same places on previous nights. The only apparent difference during the daytime between these localities and Santa Ana, where they were found resting during the day, is that at Lange and Altagracia the places searched were not as protected from breeze as at Santa Ana. It is believed that wind rather than relative humidity was the controlling factor in determining resting places. At Santa Ana the anophelines remained in small numbers throughout the day, thus being subjected to marked changes of relative humidity. At Lange and Altagracia, the anophelines departed early in the morning while the R. H. was still high (80 per cent at 8 a. m.).

D. MOSQUITO NET-CYCLOHEXANONE SPRAY.

This system was used at Vaqueria Asad, over the same places where anophelines had been previously obtained by sweeping. A total of 85 runs were made, and approximately 2,130 square feet of ground were covered. The cyclohexanone was applied twice during each run, with a waiting period between. In all these trials the only anopheline collected was 1 male *grabhami*. The adult population was low during this period—under 10 *albimanus* per overnight collection by electric light trap.

Discussion of Part III

The limited results obtained from the studies completed do not permit any definite conclusions to be drawn. The finding of *albimanus* in short grass under cocoanut palms and citrus trees is of interest, but in many portions of the island, where high populations have been collected by various types of traps, these situations do not occur. The findings of individual in open pasture land might suggest that the species rests indiscriminately in vegetation. As a large variety of ecological situations at or near ground level was examined, it is believed that further work should include examination of tree tops and other high vegetation.

Discussion and Summary—Part II and III—J. M. Henderson

DISCUSSION

The investigations described in these papers throw some light on the preferred diurnal resting places of *A. albimanus*, but they may be appraised as having only qualitative significance. The evidence points to the choice of dispersed diurnal resting places by this species, but is inconclusive due to the small number of individuals found. The very small number of *A. albimanus* collected in the San Vicente pasture does not preclude the possibility that a large, aggregate number of this species was

actually resting in this area at the times positive collections were made. The total area of pasture within easy flight distance from blood sources is in the order of 550,000 square feet. Seven female *albimanus* were captured on five different dates, an average of 1.4 per day. The mean area examined under bednet for these five positive days was 232 sq. ft., hence the collection rate per thousand square feet was 6.03. Applying this rate to the total pasture area, as many as 3,400 individuals could have been resting in this habitat area during each "positive" day. Since the data are too limited to be significant, it is considered pointless to extend these computations to include negative days.

It is considered that the significance of pasture grass and other low, dense vegetation as a possible diurnal resting place cannot be properly evaluated until a technique has been developed and a project force organized, that will permit making effective collections regularly and frequently over a minimum area of 1,000 sq. ft. daily.

SUMMARY

Numerous attempts were made to determine the preferred diurnal resting places of *A. albimanus* in Puerto Rico, using a variety of technics—visual search, beating and sweeping, and the use of an excitant or irritant under a bednet enclosure. Numerous ecologic situations were investigated. As a result of five months of sustained, systematic effort by Rehn, assisted by Mateo Serrano, only eight *albimanus* specimens were collected near blood sources. These were in pasture grass. Sixty-one adult *albimanus* specimens were collected by Maldonado by sweeping low vegetation under cocoanut palm shade, of which 26 were females. Four other specimens of this species were collected at other locations in the daytime. One hundred and twenty-two *grabhami* of both sexes were collected by Maldonado from daytime resting places, mainly in association with *albimanus*. Two *grabhami* and one *vestitipennis* females were recovered by Rehn from pasture grass.

The use of a collecting technic for capturing mosquitoes seeking shelter in low grasses is described.

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SUMARIO

Se hicieron numerosos intentos para determinar los lugares preferidos de reposo diurno de *A. albimanus* en Puerto Rico usando una variedad de técnicas-inspección ocular, golpeado y barrido y el uso de un excitante o irritante dentro de un mosquitero. Se investigaron numerosas condicio—nes ecológicas. Como resultado de un esfuerzo sostenido y sistemático durante cinco meses desplegado por Rehn con la asistencia de Mateo Serrano solamente ocho especímenes de *A. albimanus* fueron capturados cerca de fuentes de sangre. Esto ocurrió en potreros. Sesenta y un especímenes de adultos de *A. albimanus*, entre los cuales se contaron 26 hembras fueron obtenidos por Maldonado barriendo la vegetación pequeña bajo la sombra de cocoteros. Otros cuatro especímenes de esta especie fueron capturados en otros lugares de reposo durante el día. Ciento veinte y dos *grabhami* de ambos sexos fueron capturados por Maldonado en lugares de reposo durante el día principalmente en asociación con *albimanus*. Dos *grabhami* y un *vestitipennis* hembra fueron recogidos por Rehn en grama de pastoreo.

Se describe el uso de una técnica para capturar mosquitos que buscan abrigo en as pajas bajas.

OBSERVATIONS ON THE FLIGHT AND LONGEVITY IN NATURE OF *ANOPHELES ALBITARSIS DOMESTICUS*

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Malaria transmission takes place in many areas in the State of São Paulo, Brazil. (Fig. 1). In a large part of the plateau and interior as well as in some areas in the lowlands near the foothills of the Serra do Mar, *Anopheles* (*Nyssorhynchus*) *darlingi* is the most important vector responsible for the existence of the disease. From the middle of the Atlantic Coast (Peruibe) up to the northern part, *Anopheles* (*Nyssor-*

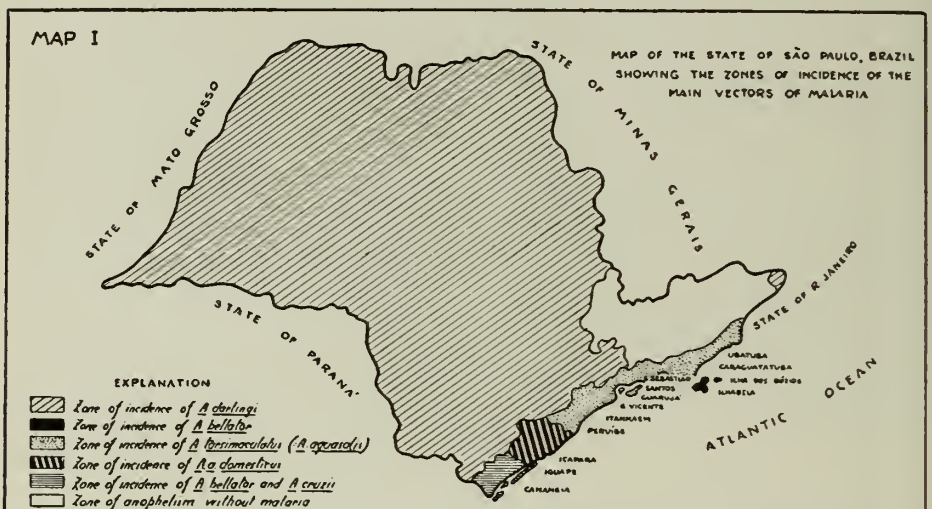

FIG. 1

hynchus) *tarsimaculatus* (= *A. aquasalis*) is the principal vector except in some areas where species of the subgenus *Kerteszia* have been incriminated in the transmission of the disease. Recently, Aldino Schiavi, who was working on the isalnd of Buzios, which is situated some 25 km. from the northern part of the sea coast, collected only *Anopheles* (*Kerteszia*) *bellator* in a place where there had been more than 100 cases of autochthonous malaria. That species was also found to be transmitter of malaria on Ilha Bela by Firmino O. Lima and Aldino Schiavi.

Correa, Lima and Ramos (1948) observed that both *Anopheles cruzii* and *A. bellator*, which belong to the subgenus *Kerteszia*, were transmitting malaria in the village of Cananéia, which is situated on the coast in the extreme south.

Iguape, another village near the coast about 40 kilometers north of Cananéia, presents a different malarial problem because there *Anopheles albitarsis domesticus*, a subspecies described by Galvão and Damasceno (1944) from material received from the States of Para and Rio de Janerio, Brazil, was found to be the vector. David

Coda, co-author of this paper, had suspected this fact as early as 1939, as he captured *A. a. domesticus* (then called *A. albitarsis*), in houses. He did not find breeding places of that species in the village so that he suspected that it probably came from Ilha Comprida which lies in front of Iguape on the other side of the Mar Pequeno. Later Aldino Schiavi (1945), from a series of investigations carried out in that area, confirmed those suppositions when he found *A. domesticus* naturally infected. The specimens which he examined had been caught indoors. Neither Coda nor Schiavi found breeding places near Iguape itself and they suspected that adults had come from the island as they had found a few larvae there.

Their observation that the main breeding places of *A. a. domesticus* are on Ilha Comprida was confirmed. In the village of Iguape and on Ilha Comprida we have found the following:

VILLAGE OF IGUAPE		ILHA COMPRIDA	
Species	No. of larvae	Species	No. of larvae
A. a. domesticus	46	*A. a. domesticus*	5,032
A. argyritarsis	1	*A. maculipes*	4
A. eiseni	102		
A. intermedius	149	Total	5,036
A. noroestensis	11		
A. oswaldoi	319		
A. strodei	1		
A. triannulatus davisi	1		
Total	630		

Total of breeding places found in the village	66
Positive for *A. a. domesticus*	6
Total of breeding places found on Ilha Comprida	19
Positive for *A. a. domesticus*	18

The foci in the village were represented by borrow-pits, brick-pits, ditches, swamps, ponds and springs; those of Ilha Comprida by streams, ponds and borrow-pits. Streams are the permanent breeding places of *A. a. domesticus* on the island because all the other types dry up during the winter months.

In March 1947 observations were started in order to find out whether some specimens of *A. a. domesticus* flew from Ilha Comprida to the village of Iguape and whether others came from the village to the island. Mosquitoes were stained and released in order to investigate this problem.

A large number of experiments on the flight and dispersion of marked mosquitoes have been carried out in the past. (Eyles 1944)

METHODS AND RESULTS

In order to determine whether *A. a. domesticus* could fly from Ilha Comprida to the village of Iguape, mosquitoes were stained with a 2 per cent watery solution of methylene blue. For this purpose the mosquitoes were put into a bobbinet cage and

applied for the solution with a small sprayer. Later the presence of stain was verified on mosquitoes recaptured by dropping on them a small amount of the following solution:

Glycerin	3 parts
Alcohol	2 parts
Chloroform	2 parts

The technique of staining requires special care because the anophelines can easily be wetted too much so that they can no longer escape from the bobbinet cage.

On March 17, 1947, the capture of adults and larvae of *A. a. domesticus* was started on Ilha Comprida in front of the village of Iguape. Four hundred and thirty-nine adults were caught inside houses and 104 with a light-trap. In addition 2,277 females and 1,800 males were bred from larvae collected in several streams on the island. A total of 4,700 specimens were stained which included 1,880 males and 2,820 females. The release of these mosquitoes was carried out on the island, close to a house located about 1,500 meters from the village of Iguape.

The staining and releasing of marked mosquitoes began on March 17 and ended on May 13, 1947. Recaptures were initiated on March 22 in the village of Iguape, with animal bait, with a trap, and inside houses along the Mar Pequeno lagoon, just in front of Ilha Comprida. Out of a total of 750 anophelines taken at Iguape from March 22 to December 24 only two stained females were encountered. The first was found on April 9 (18 days after release) and the second on April 11 (20 days after release), both inside houses. No males were recaptured.

During 1948 attempts were made to find out whether *A. a. domesticus* could travel from Iguape to Ilha Comprida. For that purpose a metallic red bronzing powder was used, which was applied with a small atomizer. Anophelines were first caught inside houses from February 2 to March 19 and recaptures were initiated on March 14 on Ilha Comprida.

A total of 2,240 marked mosquitoes was released at Iguape and 26 stained specimens (1.1 per cent) were recaptured on Ilha Comprida out of a total of 713 collected. All of them were found in the interior of houses located about 1,500 meters from the village.

DISPERSION AND LONGEVITY IN NATURE

There are very few published observations on the longevity of anophelines in nature as compared with observations carried out on flight distance.

Barber and Hayne (1924) observed the survival of *A. quadrimaculatus* 21 days after its staining. Ivanova (1942), in Russia, recaptured females of *A. maculipennis* which had been stained 36 days before. These are the only two references that we have encountered in the literature concerning the length of survival of anophelines under natural conditions.

At the beginning, the purpose of the investigations was to find out if the specimens of *A. a. domesticus* released on the island could be recaptured in the village of Iguape and vice versa. Later by studying the geographical distribution of the subspecies in the village of Iguape and recording all the dwellings for subsequent spray

treatment with DDT we hoped to get some data concerning the dispersion and longevity of *A. a. domesticus* in nature.

Thirty-eight stained mosquitoes were found among a total of 2,026 anophelines captured after the stained insects had been released. These included 713 from Ilha Comprida and 944 from the village of Iguape itself. The number of marked insects recaptured amounted to 1.69 per cent of the total of 2,240 originally released.

Stained mosquitoes were found on Ilha Comprida, in the village of Iguape, as mentioned before, and also at Rossio, Barra do Icapara (a village 12 kilometers east of Iguape) and at two other points 9 and 13 kilometers west of Iguape. Only a few captures were made on the island in front of Iguape. If they had been carried out more intensively the results might have been different and the number of recaptured

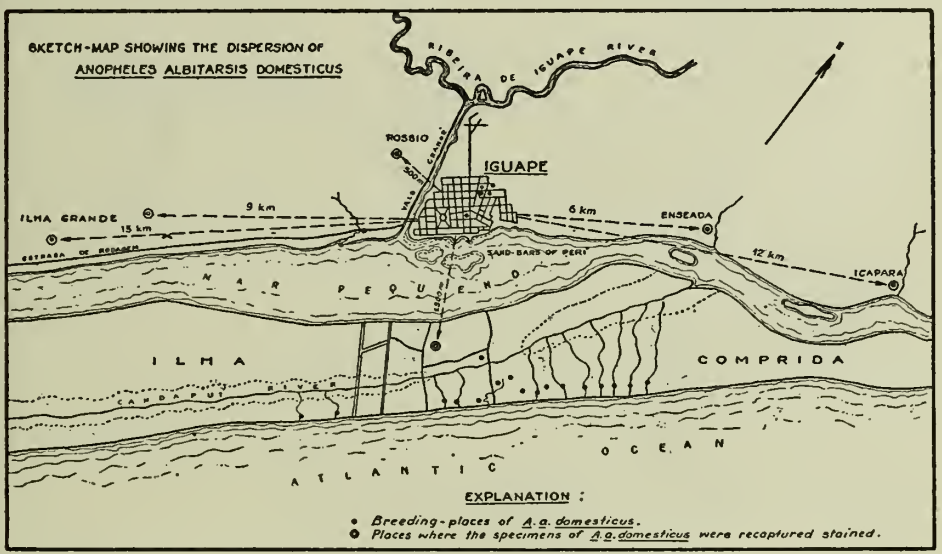

FIG. 2

mosquitoes larger. Although the numbers are not great it can at least be stated that the dispersion of *A. a. domesticus* occurred up to a distance of 12 to 13 kilometers. It is believed that this was active dispersion because passive transportation by vehicles could only have occurred from the east to the west, where there is a road. The number of vehicles, however, is very small. Maritime traffic is carried on by small boats.

This study emphasizes the necessity of using residual DDT in all dwellings situated within a radius of 20 kilometers from Iguape, as infected mosquitoes are able to carry malaria from Iguape to Ilha Comprida, Barra do Icapara and other places.

This species can fly for at least 1 kilometer without resting, as that is the width of the Mar Pequeno lagoon. In front of the village of Iguape there are some sand bars on which a plant called "piri" (*Scirpus riparius* family *Cyperaceae*) grows. It appears that mosquitoes do not rest on these sand bars.

The last group of mosquitoes to be stained was released on March 19 and the

calculation of the natural survival time was made from this date. The age of adult mosquitoes when originally captured was unknown.

The first stained anopheline was recaptured on Ilha Comprida on March 22, 3 days after the last staining. The largest sample numbering 17 individuals was collected indoors at the same place on May 20, 60 days after release. Two additional specimens were taken, one on June 15 on Ilha Comprida, and the other on August 23 in the village of Iguape.

During these studies the predominant winds blew from the east and the south.

SUMMARY AND CONCLUSIONS

Observations were carried out on flight range and natural longevity of *Anopheles albitarsis domesticus* near Iguape, State of São Paulo, Brazil, where this anopheline is the malaria vector.

The results of these investigations were as follows:

1) *A. a. domesticus* can cross Mar Pequeno lagoon the width of which is about 1,000 meters from Ilha Comprida to the village of Iguape. This flight was demonstrated by staining 4,700 mosquitoes with methylene blue and releasing them on the island. Two stained females were recaptured in dwellings in the village of Iguape.

2) This anopheline also flies across the bay from Iguape to Ilha Comprida probably in order to return to its preferred breeding places. Using a red bronzing powder 2,240 mosquitoes were stained and released at Iguape and 26 marked females (1.16 per cent) were recaptured on the island.

3) At other places, including Iguape, 12 additional marked anophelines were taken, having dispersed for distances up to 12 and 13 kilometers.

4) Seventeen out of the original 2,240 marked mosquitoes were recaptured on Ilha Comprida 60 days later.

5) We conclude from these experiments that it is necessary to spray DDT not only in the localities to be protected but also in all dwellings situated within a radius of 20 kilometers.

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